Monosodium Glutamates #39

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April 9, 1975

Dr. George W. Irving
Research Associate
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Dear Dr. Irving:

Following our recent conversation, and our submission of data to you as described in my letter of March 11th, I attach for the use of the committee three additional documents.

First, please find reprints of a paper entitled: "Monosodium Glutamate Studies in Four Species of Neonatal and Infant Animals," by B. L. Geur, et al. This paper recently appeared in Food and Cosmetics Toxicology..

In addition, Dr. Ann Reynolds of the University of Illinois has recently made available to me a preprint of a paper involving MSG, sodium chloride and sucrose and their effect on the brains of neonatal mice. This paper has recently been accepted for publication by Comparative Neurology.

Finally, I attach a preprint of a paper in press at Toxicology by Professor Kigwood on the consumption of Glutamic and Aspartic acids. Although Professor Kigwood uses total, not free amino acids, in his calculations, I believe it worthwhile to compare levels of amino acids liberated on digestion of proteins versus those ingested in "free" form.

We hope the SCOG's Committee will find these three papers of value.

Sincerely,

Chairman

A. G. Ebert, Ph.D./mtt/2/11

cc: Dr. Ann Reynolds
Mr. Jennie Peterson
IOTC Members

Fd Cosmet. Toxicol. Vol. 13, pp. 7–14. Pergamon Press, 1975. Printed in Great Britain.

MONOSODIUM GLUTAMATE STUDIES IN FOUR SPECIES OF NEONATAL AND INFANT ANIMALS

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Abstract—As an extension of our previous work, the effect of both intragastric and sc administration of monosodium glutamate (MSG) was studied in four species of neonatal animals (mice, rats, beagle dogs and cynomolgus monkeys). Control groups were dosed with sodium chloride, sodium gluconate, potassium glutamate and distilled water. A uniform dose level of 1 g/kg body weight (10 ml of a 10% aqueous solution/kg) was used. Animals were killed at 3, 6 or 24 hr (and in the case of dogs also at 52 wk) after dosage. Several monkeys were also dosed orally with MSG or sodium chloride at 4 g/kg.

No adverse effect was observed on growth, appearance or behaviour in any of the species. Examination of multiple sections of the brain, eyes and pituitaries by light microscopy revealed no pathological alteration. Failure to observe in the arcuate nuclei of the hypothalamus the lesion described by Olney (*Science, N.Y.* 1969, **164,** 719) is discussed, in relation particularly to the 1 g/kg dosage level, which is many times the estimated maximum human dietary level.

INTRODUCTION

Olney (1969) initially reported a neuropathological effect of monosodium glutamate (MSG) upon the brain of the mouse. Although lesions were found in adult mice given sc doses of 5–7 g/kg, infant mice were more sensitive. Mice of 2–9 days of age were given a single sc injection (dosage ranging from 0·5 to 4 g/kg body weight) and were killed 1–48 hr after dosing. Light-microscopic examination revealed acute neuronal necrosis (intracellular oedema and nuclear pyknosis) preferentially affecting the paramedian areas bordering on the roof and floor of the third ventricle, particularly the preoptic and arcuate nuclei of the hypothalamus and neurons of the median eminence. Long-range effects, in mice given daily sc injections for the 10 days after birth (2·2–4·2 g/kg body weight) and observed to 9 months of age, included skeletal stunting, marked obesity with increased body weight, lethargy, poor pelage and female sterility. It was postulated that these findings comprised a "complex endocrine disturbance" related to the neonatal disruption of neuronal development of "regions of the brain thought to function as neuroendocrine regulatory centers".

The report also raised a question of a "risk to the developing human nervous system by maternal use of MSG during pregnancy". In a subsequent paper (Olney & Sharpe, 1969), the risk consideration was broadened to include human infants fed glutamate-enriched diets. To support such concern, the paper presented a description of acute neuronal necrosis in the periventricular-arcuate region of the hypothalamus of an infant rhesus monkey killed 3 hr after a single sc injection of 2·7 g MSG/kg given 8 hr after birth.

During this time the present study was planned and work was initiated. It was intended to provide information on several points, namely the question of whether the neuronal effects of single doses of MSG are reproducible, the influence of the route of administration (intragastric (ig) versus sc), the possible role of the degree of ionization of the material or

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the concentration of the sodium ion, possible species differences in the neuropathological effect of MSG and the influence of the age of the animal. In the latter connexion, the neonatal period (about 3 days after birth) was compared with the period when the lactating animal no longer depends completely on maternal milk and begins to take solid nourishment, a period about 12 days after birth for mice and rats and 35 days after birth for dogs. The MSG dose level used in the present study, 1 g/kg body weight, was high not only in relation to any conceivable intake by the human adult but also in relation to the 0·1–0·6 g MSG that might have been present in 4·5 ounces of baby foods formulated before the discontinuance of glutamate addition to baby foods by producers. It was considered reasonable therefore to focus on the 1 g/kg dose level in the present multi-species experiment.

EXPERIMENTAL

Materials. To evaluate the role of both the sodium and glutamate moieties of MSG and study the possible influence of ionization, four materials were tested, namely MSG, sodium chloride (completely ionizable), sodium gluconate (incompletely ionizable) and potassium glutamate, together with distilled water as a control. Consideration was given to the use of molar or molal equivalence, but it was decided to administer all test materials as sterile 10% (w/v) aqueous solutions, with animals receiving a constant dosage of 10 ml/kg body weight (equivalent to 1 g/kg).

Animals. The animals used were C57BL/6J infant mice, obtained from inhouse breeding of parent mice purchased from a commercial supplier, infant rats of the FDRL strain, beagle pups from the FDRL breeding colony and infant cynomolgus monkeys.

Treatment schedules. Both ig and sc routes of administration were used for each age group and species. Animals of each of the four species were dosed at or close to 3 days of age, and, in a second series, at ages corresponding to the period of introduction of 'solid' foods into the diet, as described above. A uniform dose level of 1 g/kg was used for all animals and test materials, except in the case of some monkeys, as indicated below.

Mice were divided into 20 groups of five, dosed either ig or sc at 3 or 12 days of age and killed 24 hr after dosage. Ten groups of eleven 3-day-old rats were also dosed by both routes, three rats from each group being sacrificed after 6 hr and eight after 24 hr. In addition, ten groups of five 12-day-old rats were similarly dosed and killed after 24 hr.

Three-day-old beagle pups were dosed by both routes in groups of six, one animal from each group being killed after 3 hr, two after 24 hr and three after 52 wk. The last three were observed daily and weighed monthly and at termination they were autopsied, the organs were weighed and femur length was measured (as an index of skeletal growth). Thirty additional dogs were treated (by sc and ig routes) at 35 days of age, one in each group of three being killed after 3 hr and two after 24 hr. Five additional groups of three 35-day-old pups were given gradually increasing ig doses (2·2–4·4 g/kg) over a 10-day period, according to the protocol of Potts, Modrell & Kingsbury (1960). Their progress was followed for 48 wk, at which time electroretinograms were recorded and examined. They were then killed and autopsied and organ weights and femur lengths were recorded. Extensive microscopic examinations were made of the brain, eyes and pituitary.

Thirty-two 3- or 4-day-old cynomolgus monkeys were studied. Doses of all the test compounds were administered at the 1 g/kg level both ig and sc in the form of 10% solutions and, in addition, two monkeys were given orally 4 g sodium chloride/kg as a 20% aqueous solution and five received 4 g MSG/kg orally. Of the latter group, one received the dose

in baby food and was killed 3 hr later, and the others were dosed with a 20% aqueous solution, two being killed at 3 hr and one each at 6 and 24 hr. The sodium chloride-treated monkeys were killed 3 and 24 hr after dosing.

Histopathology. Initially, rodents were anaesthetized with ether, the head was sagitally bisected and needle biopsies of the right ventral hypothalamus were taken immediately and fixed in 3% glutaraldehyde in phosphate buffer. Both halves of the brain were fixed in 10% neutral buffered formalin. All the mice and some rats and dogs were treated in this way. Subsequently the animals were anaesthetized (with ether for rats, Surital for dogs and Sernylan for monkeys) and cannulated into the ascending aorta through the left ventricle, and the brain was fixed by perfusion with 3% glutaraldehyde in phosphate buffer following a brief perfusion with heparinized saline or phosphate buffer. The glutaraldehyde perfusion time was about 10 min for rats and 15–20 min for dogs and monkeys. The fixed brain was removed and sectioned transversely through or near the pituitary stalk, and a 1-mm transverse slice just caudal to the stalk was removed, additionally fixed in glutaraldehyde and then stored in cold phosphate buffer for possible electron microscopic examination. The brain was stored in 10% neutral buffered formalin prior to further processing.

Gross examination of other organs (and storage of organs or samples in formalin) was carried out in the monkeys and in the dogs, other than those dosed when 3 days old and killed after 3 or 24 hr.

Examinations by light microscopy were made of 6-μm sections of paraffin-embedded tissue stained with haematoxylin and eosin. For some of the earlier studies, the left half of the brain of the mice and rats was sectioned sagitally. This was soon replaced by transverse or coronal sectioning for all other animals, and later coronal sections were prepared from the right half of the brain of many of the rodents studied earlier. Spaced sequential sections were taken throughout the hypothalamus, spanning the region from the preoptic level to the interpeduncular zone or premammillary level. The average number of sections examined for each animal was 30 for mice and rats, 20 for dogs and 20–30 for monkeys. In addition, light-microscopic examination was carried out on sections of certain endocrine organs from the 30 dogs dosed once at 3 days of age and followed for 52 wk.

Nearly all the slides were examined by two pathologists, one of whom read them blind.

RESULTS

In essentially every instance, microscopic examination of brain sections of animals aged 3–5 days showed occasional small neurons with nuclear pyknosis and a more frequent, but still sparse, scattering of small cells showing nuclear pyknosis or karyorrhexis with cytoplasmic eosinophilia and without any glial or inflammatory reaction. This kind of cell was widely distributed in the brain but was sometimes a little easier to find (presumably being more numerous) in the periventricular-arcuate region of the hypothalamus. However, these cells exhibited no apparent swelling (typically they are smaller than most of their neighbouring cells) and they were seen in the brains of animals of all groups.

Neuronal necrosis of the hypothalamic arcuate nuclei, as described and illustrated by Olney (1969), was not identified in any animal of any group, notwithstanding the variations in techniques of autopsy, fixation and sectioning. Only in one animal, a rat, dosed with 1 g MSG/kg at 3 days of age and killed 24 hr later, was an area seen that closely

resembled the lesion described by Olney (1969). In this animal, an area in the median eminence contained cells showing slight nuclear pyknosis and prominent vacuolation. However, most of the vacuolation appeared to be intercellular and was possibly an artefact, and the area was not believed to be a lesion attributable to the effect of MSG.

Particular attention was paid to the brain sections of the five monkeys dosed with 4 g MSG/kg and re-examination of the sections from most of the animals dosed with MSG or potassium glutamate revealed no changes with the reported features of MSG-induced necrosis, or even showing any difference from the other groups.

Table 1. *Body weights of dogs maintained to maturity following a single 1 g/kg dose of MSG or other test material at 3 days of age*

Test compound	Dosage route	Dog no. and sex	Body weight (kg) at wk					
			0*	4	12	24	36	52
MSG	Oral	3201 F	0·47	1·21	4·6	8·6	10·0	10·2
		3202 F	0·36	1·10	2·9	6·6	7·6	7·7
		3203 M	0·44	1·21	5·4	9·5	11·1	11·1
		Mean...	0·42	1·17	4·3	8·2	9·6	9·7
	Sc	3207 F	0·51	1·22	3·9	7·4	8·3	8·7
		3208 M	0·54	1·27	6·4	10·2	16·1	16·6
		3209 M	0·60	1·35	5·7	9·2	13·5	15·7
		Mean...	0·55	1·28	5·3	8·9	12·6	13·7
NaCl	Oral	3213 F	0·56	1·24	3·9	7·8	8·7	11·3
		3214 F	0·33	1·05	3·0	7·5	8·5	9·0
		3215 F	0·48	1·20	5·1	8·7	10·6	11·0
		Mean...	0·46	1·16	4·0	8·0	9·3	10·4
	Sc	3219 F	0·52	1·20	3·6	8·0	8·7	9·7
		3220 M	0·42	1·16	5·6	8·6	11·6	12·0
		3221 M	0·61	1·32	6·1	9·3	12·5	11·7
		Mean...	0·51	1·23	5·1	8·6	10·9	11·1
Na gluc	Oral	3225 F	0·54	1·25	3·6	7·9	8·8	10·3
		3226 F	0·27	1·09	3·8	7·6	8·2	8·7
		3227 M	0·46	1·29	6·1	10·4	15·3	15·1
		Mean...	0·42	1·21	4·5	8·6	10·8	11·4
	Sc	3231 F	0·48	1·10	3·9	7·5	8·9	9·1
		3232 F	0·46	1·12	4·8	9·0	11·3	12·0
		3233 M	0·62	1·30	6·2	9·4	15·4	16·4
		Mean...	0·52	1·17	5·0	8·6	11·9	12·5
MKG	Oral	3237 F	0·37	1·10	4·0	7·9	7·9	7·7
		3238 M	0·48	1·23	4·5	9·2	12·0	11·6
		3239 F	0·61	1·36	4·8	9·6	12·0	12·4
		Mean...	0·48	1·23	4·4	8·9	10·6	10·6
	Sc	3243 F	0·56	1·31	5·1	9·5	11·3	11·3
		3244 F	0·40	1·10	3·7	7·5	9·9	9·7
		3245 F	0·54	1·24	4·8	8·6	9·7	9·4
		Mean...	0·50	1·22	4·5	8·5	10·3	10·1
Water	Oral	3249 F	0·55	1·15	3·5	7·6	8·9	9·6
		3250 F	0·25	1·12	3·5	7·2	8·2	8·7
		3251 F	0·35	1·11	4·8	8·5	8·8	8·6
		Mean...	0·38	1·13	3·9	7·8	8·6	9·0
	Sc	3255 M	0·55	1·28	5·6	9·4	14·3	15·1
		3256 M	0·53	1·21	4·9	8·4†	—	—
		3257 M	0·37	1·15	4·9	8·8	12·8	14·1
		Mean...	0·48	1·21	5·1	8·9	13·6	14·6

MSG = Monosodium glutamate Na gluc = Sodium gluconate MKG = Monopotassium glutamate
*Initial body weight.
†Death ensued before the next weighing.

Microscopic examination of the eyes of these animals, and scrutiny of the electroretinograms of neonatal dogs dosed serially according to the procedure of Potts *et al.* (1960) and Cohen (1967), failed to reveal any effect of MSG or any significant difference between the groups.

Gross and microscopic examination was carried out on the pituitary gland, ovaries, uterus and mammary glands of the 30 dogs dosed once at 3 days of age and observed for 52 wk. No abnormalities or differences between groups were found. Body weights, recorded monthly during the 52 wk these animals were followed, showed no evidence of an effect of any treatment (Table 1).

In the additional group of 15 dogs dosed for 10 days, starting when they were 35 days old, and autopsied at 48 wk, no unusual or treatment-related changes were found in the gross examination or weights of organs (gonads, liver, spleen, kidneys, adrenals, thyroid, pituitary, heart and brain), in femur lengths, in electroretinograms or in the microscopic examination of the brain, eyes and pituitary.

Slight regurgitation of mucus was noted in one of the sodium chloride-treated monkeys and at autopsy (24 hr after dosing) the stomach mucosa appeared reddened. The other member of this pair was sacrificed 3 hr after dosing. The stomach and upper small intestine contained blood and the mucosa appeared haemorrhagic. No such findings were seen in the animals on MSG.

DISCUSSION

During the past few decades, MSG has been the subject of a number of toxicological and pharmacological investigations in several animal species, including man. Retinotoxic properties were reported by Lucas & Newhouse (1957), who described degeneration of the ganglion-cell layer and failure of formation of the inner nuclear layer of the retina. Inhibition of formation of the inner retinal layers was confirmed by Potts *et al.* (1960), following ip dosing of MSG to infant mice at daily increasing levels for 2–17 days. These authors proposed that, in the mouse, MSG repressed an essential enzyme needed for the development of the inner retinal layers. Cohen (1967) reported the destruction of axons in the optic nerves of 2-month-old mice given MSG as neonates (2·2–4·2 g/kg on days 1–10 after birth).

The present investigation was prompted by the report that sc administration of high doses of MSG (0·5–4 g/kg) exerted a neurotoxic effect on several regions of the brain of neonatal rodents (Olney, 1969) which, as previously mentioned, was interpreted as suggesting a neurological hazard to the developing human foetus "by maternal use of MSG during pregnancy". The relevance of the original Olney (1969) study to the safety of MSG as used in foods was questioned (Blood, Oser & White, 1969) on the grounds of the age of the test animals, the routes of administration, the magnitude of the doses and the lack of adequate controls. Several additional correspondents (Lowe, 1970; McLaughlan, Noel, Botting & Knipfel, 1970; Zavon, 1970) questioned the work of Olney (1969) because (a) data were lacking on the response of control mice to the solvent alone or to equivalent amounts of sodium chloride or the salts of other amino acids; (b) no blood-level data were gathered; (c) only one primate was used; and (d) the levels of MSG used in the neurotoxicity studies seemed excessively high in relation to potential human ingestion. Olney & Sharpe (1969, 1970) later supplied some of the information lacking in the original report, as well as additional data which supported their conclusion about the effect of high doses of MSG in the infant animal.

Adamo & Ratner (1970) studied both acute and long-range effects in rats given a single sc injection of 4 mg MSG/kg when 3 or 4 days old and were unable to find any evidence of an adverse effect of glutamate on neural morphology or the reproductive system.

Arees & Mayer (1970) reported that approximately 95% of their MSG-treated mice, given a single sc or ip injection (at a dosage of 2 or 4 g/kg for infant mice and 6–10 g/kg for adult mice) and killed 3–72 hr later, showed lesions in the arcuate nuclei of the hypothalamus. However, the lesions were smaller than those reported by Olney (1969) and consisted primarily of degenerating microglial cells, the perikarya of neurons being unaffected.

Olney & Ho (1970) reported brain damage in 10–12-day-old mice killed 3–6 hr after receiving a single oral dose of MSG (dosage 0·5–2 g/kg) or monosodium aspartate (dosage 1 g/kg). The arcuate nucleus of the hypothalamus showed well-demarcated lesions, in which the number of necrotic neurons in each cross-section of the arcuate nucleus was dose-related. Neuronal cytopathology was not found in controls, which included some animals treated with "massive" doses of sodium chloride or "large amounts" of sodium bisulphite or glutarate.

Reynolds, Lemkey-Johnston, Filer & Pitkin (1971) found hypothalamic lesions (neuronal necrosis) in the newborn mouse following oral administration of MSG, but in their study of infant monkeys no hypothalamic changes could be found in animals sacrificed 6 hr after ig administration of 1–4 g/kg. Abraham, Dougherty, Golberg & Coulston (1971) distinguished two types of lesions in the arcuate nuclei of mice given MSG, ig administration involving glial cells and sc administration involving neuronal cells. The incidence of lesions was higher at a dosage of 4 g/kg than at 1 g/kg, and higher with sc than ig administration. In a similar examination in which the hypothalamus of four infant monkeys given 4 g MSG/kg (two orally and two sc) was compared with that in control infant monkeys, no effect was found. The authors suggested that the species difference in susceptibility to large doses of MSG may relate to the disparate permeability of the blood–brain barrier in neonatal animals consequent upon different degrees of myelination of the central nervous system. The rate of intestinal absorption was suggested as a limiting factor in the hypothalamic effect in mice.

In the studies here reported, involving the ig or sc administration of single 1 g/kg doses of MSG to rats and mice 3 or 12 days of age or to dogs 3 or 35 days of age, and killed 3 hr (dogs), 6 hr (rats) or 24 hr (rats, mice and dogs) after dosage, no hypothalamic pathology was observed. Single 4 g/kg ig doses given to five infant monkeys were likewise negative. Growth, appearance and behaviour appeared normal and gross and microscopic examination at autopsy revealed no abnormalities in the brain, eyes, pituitary, ovaries and uterus of dogs given a single dose at 3 days of age and observed for 52 wk. No evidence of pathological change was found in electroretinograms or in the eyes or brain of dogs given increasing daily doses for 10 days from 35 days of age and followed for 48 wk.

Several possible reasons may be suggested for this failure to observe neurotoxic effects with MSG. The hypothalamic lesion described by Olney (1969) may be so slight as to be difficult to find by any technique short of examination of serial sections of the entire ventral hypothalmus. This study was not designed to detect and quantitate lesions of such minute degree. However, if even very small lesions had been present in these animals, it seems improbable that the examination of periodic or spaced sections would miss them every time. The thickness of light microscopic sections and the stain used may be important in the optimal visualization of the lesion. This study used 6-μm sections stained with

haematoxylin and eosin, whereas Olney & Sharpe (1969) used 1-μm sections stained by the Richardson method (Richardson, Jarett & Finke, 1960). Considering the light microscopic features of MSG neuropathology (swollen cell, vacuolated cytoplasm and nuclear pyknosis), it seems to us unlikely that these effects would be shown by only one of these stains.

It should be noted that in a more recent report (Olney, Sharpe & Feigin, 1972) the single 1-wk-old monkey dosed orally at 1 g/kg showed "tiny lesions" consisting of acutely necrotic neurons "almost exclusively in rostral subventricular nucleus". At doses of 2 or 4 g/kg, such lesion sites were more conspicuous and somewhat greater in number.

The histological technique for fixing tissues is of crucial importance in electron microscopy, but is not so critical in light microscopy, at least not for the features claimed to be induced by high doses of MSG. Some of the initial work here reported was done with ordinary formalin fixation; when the perfusion method was first used, the technique failed in occasional animals. Nevertheless variations in fixation procedures provided ample opportunity for revealing the presence of hypothalamic lesions, if in fact they were present. The possibility that inadequate fixation might somehow reverse the lesions so that necrotic cells would appear normal is hardly tenable.

Oral or ig dosage is subject to criticism on the grounds of possible loss of test material by regurgitation or vomiting (either from an excessive dosage volume or an emetic action of the material). The absence of MSG effects in this study cannot be due to this factor, however, because lesions should then have been found in animals dosed sc.

No adverse effect on neurological or hepatic function was observed by Bazzano, D'Elia & Olsen (1970) in human adult males receiving as much as 137 g glutamic acid daily for periods ranging from 14 to 41 days, nor in gerbils fed diets containing glutamate at a level equivalent to 30 g/kg body weight/day. These authors, like others, suggest that the central question is how much of the fed or injected MSG actually crosses the blood–brain barrier in the neonatal animal and whether the rate of development of the blood–brain barrier in rodents or primates is comparable to that in the human infant. There is no doubt that passage across the barrier is a function of the blood level and, in turn, of dose and concentration.

Though the existence of a blood–brain barrier has been questioned, the evidence concerning a wide series of active agents shows that, with maturation, there is a "sparing" action (i.e. a lower central sensitivity to toxicants) indicative of the development of such a barrier. Its specific time of appearance is largely determined by functional tests, but it has been shown to be present when the animal begins to ingest the 'solid' food offered in the maternal diet.

Several reports of induced hypothalamic lesions involved a 4 g/kg daily dose, whereas 1 g/kg was used in most of these studies. The higher dose would be equivalent to about 15 g for a newborn human infant. The administration of massive dosages to 1–3-day-old rats places a substantial toxic stress on the neonatal organism when considered in the light of the fact that metabolic enzyme systems are still in the stage of development during the period of lactation.

The present study fully supports the conclusion of the Food Protection Committee (1970) that the risk associated with the use of MSG in infant food is extremely small and that, except for persons individually sensitive to MSG, foods containing the flavour enhancer present no hazard for older children and adults.

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IS THE CONSUMPTION OF L. GLUTAMIC ACID AND OF L. ASPARTIC ACID LIABLE TO CAUSE DAMAGE TO HEALTH

In 1970, J.W. Olney, MD, Associate professor of psychiatry, Washington University Medical School, published observations on the damage in the brain of ten day old mice produced by oral intake of glutamic or aspartic acids. He claims that the health of young children could be adversely affected by the consumption of aspartame ; that glutamate and aspartate are toxic and produce irreversible necrosis of hypothalamic neurons from only a single exposure. He underlines the fact that although the focus of attention in animal research was put by him on the new born animals, no one, in his opinion, has shown that older animals lack susceptibility. He believes, on the contrary, the opposite to be true. He places the threshold for brain damage from oral monosodium glutamate (*) at :

- 0.5 to 1 mg per g. in 10 day old mice
- 1 to 1,5 mg/ g in weanling, and
- 1,5 to 2 mg/g in older animals.

If a similarly gradual shift in the toxic threshold accurs with age in humans, it is reasonable, in his opinion, to assume that infants or children would be at risk from 0.5 to 1,5 mg/g and adolescents and adults from 1,5 to 2 mg/g.
The same figures correspond of course to grams per Kg body weight.

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(*) J.W. OLNEY et al., Science 1969, 164, 719 ; 166, 386 ; 1970, 167, 1017 ; Nature 1970, 227, 609 ; J. neuropathol. Exptl. neurol. 1972, 31, 464.

It must be emphasized that L. aspartic acid and L. glutamic acid are two aminoacids present in all dietary proteins, and that they are most frequently the two most abundant aminoacids in these nutrients, certainly as regards glutamic acid.
The two together form most frequently some 30 to 40% of the total load of protein in the diet.
In animal proteins the mean figure corresponds to roundabout 30%, whereas in vegetable proteins it is of the order of 39% (see appending table where the major basic food products of the diet are represented).

For the entire population of the highly industrialized countries, where the protein intake is more or less evenly distributed between the animal and vegetable proteins, with only a slight advantage of the latter on the former, it may be taken that the sum of glutamic and aspartic acids correspond all in all to some 35% of the protein supply of the diet.

Proteins are broken down into free aminoacids in the course of digestion in the gut and absorbed there through the gastro intestinal mucous membrane into the blood. The dietary supply of glutamates and aspartates is therefore abundant, and there is no reason to suspect that their eventual action on the brain might be different from that of the same aminoacids consumed as such, namely in the form of their monobasic alcali salts, provided of course that it is the L. isomers which are concerned.
Let us therefore examine the matter from the nutritional angle, and let us see first of all what the daily intake is of those two aminoacids derived from the proteins of the diet.

THE WEANING INFANT. -

At birth and shortly after birth, as long as the infant remains exclusively breast fed, it consumes the two aminoacids derived from human milk alone.

Human milk contains on an average about one g. of protein per 100 g. milk and this yields a supply of 0.282 g. of aspartic and glutamic acids (see the FAO-WHO tables of amino-acids content of food, Rome 1970, pages 132-135) (*).

A consumption of 500 ml of human milk supplies therefore 1.4 g. of aspartic and glutamic acids to the infants (0.282 x 5).

Cows milk contains on an average 3.5 g. protein per 100 g. of milk and about 34% of this corresponds to aspartic and glutamic acids, thus about 1.2 g.

The consumption of one half to one litre of milk supplies thus 6 to 12 g. of the two aminoacids to a weaned infant weighing about 5 Kg, thus some 1.2 to 2.4 g. of the two aminoacids per Kg b.w.

The toxic threshold assumed by Olney for infants (0.5 to 1.5 g/Kg) would therefore be exceeded by the intake of the two aminoacids derived from milk. The implication is that the infant consuming one half to one litre of milk would be at risk of suffering brain damage.

OLDER CHILDREN BEFORE ADOLESCENCE

(average for a ten year old child, range of 2 to 14 years of age) average body weight 32 Kg (range from 20 to 45 Kg). In fact the average figures for body weight of children in function of age differ somewhat from country to country. Those given here are valid for the Belgian population. In the USA they are somewhat higher.

(*) 101 mg aspartic acid and 181 mg glutamic acid per 100 g. of human milk. The content in protein is usually based on the total nitrogen content (x 6.25), this includes therefore nitrogen of mucopolysaccharides and other non protein nitrogenous compounds such as urea for instance. The amount of the two aminoacids (0.282 g) corresponds therefore to about 30% of the true protein content of human milk.

Olney sets the threshold of risk from an intake of aspartic and glutamic acids at the level of 0.5 to 1.5 g/Kg body weight. At the age of ten, the actual daily intake of protein averages about 1.25 g. per Kg body weight, possibly even a little more than that, and 35% of this corresponds to the daily intake of aspartic and glutamic acids, thus to at least 0.44 g. of them per Kg. b.w. (*)
Young children could therefore easily exceed Olney's lower limit for safety of these aminoacids from consumption of ordinary food. This would imply that children, especially those with above average protein intake, would be continuously at risk of brain damage.

ADOLESCENTS AND ADULTS

The average body weight for both sexes : some 60 Kg at least. For men it often reaches the figure 70 Kg and possibly 80 or 90 Kg and even more. The actual protein intake of 100 g. is frequent, possibly even more than that. This corresponds to at least 35 g. of aspartic and glutamic acids, eventually even somewhat more than that, thus some 0.6 g. per Kg b.w. at least.

According to Olney the risk of brain damage due to the consumption of these two aminoacids exists at the level of consumption of 1.5 to 2 g. per Kg b.w. In other words, this category of people would still be on the safe side, although there would not be much of a safety margin in this respect.

(*) The figure 1.25 g. protein per Kg body weight is often given as the requirement, but the actual intake is usually higher than that, approaching easily 2 g. at this age.

Such a state of affairs is to be regarded as being highly improbable. Physiologically speaking, the adult human being and the adolescents do not live that close to unsafety as regards the eventual harmfulness of the nutrients they consume when living on a normally balanced diet. It is therefore very definitely our feeling, and even our conviction in the case of the normal diet of weaned infants, that Olney's views on the matter are in need of being critically reexamined with great scrutiny, and even confirmed eventually on the basis of findings in animals made in more than one laboratory. It is anyhow always desirable that observations liable to involve important consequences in the field of public health, be confirmed in more than one research laboratory.
It is clear from these calculations that infants and children regularly consume ordinary foods containing quantities of aspartic acid and glutamic acid in the range considered by Olney to be toxic. In view of this it is essential that these claims be evaluated with great care and that the basis for them be examined most critically. It would be strange, indeed, if the ordinary food that we human beings consume throughout life were to contain quantities of two amino-acids in the near-toxic range according to Dr. Olney's predictions.

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ASPARTIC ACID CONSUMPTION DERIVING FROM ASPARTAME

The molecular weight of this sweetener which is a dipeptide is 294, and that of aspartic acid 133 (that of phenylalanine is 165).
The aspartic acid moiety of the molecule corresponds therefore to 45% of the weight of aspartame.
The sweetening potency of aspartame is of the order of 200 against 1 for sucrose.

The total human consumption of sugar per head of the population varies from country to country. In most western European countries it is of the order of 100 g. per day. In the USA and in Sweden it is closer to 150 g. If this sweetener were entirely replaced by aspartame, in the human diet, the consumption of this latter sweetener would amount to about 0.5 g. per head of the population in most European countries and 0.75 g. in the USA or in Sweden. This is however an unrealistic evaluation of what the upper limit of aspartame consumption might be, because this sweetener cannot be used in certain types of food products, namely those which require prolonged cooking in the course of their preparation.
Let us consider a level of intake of aspartame equivalent in sweetening potency to one half of the total amount of sugar consumption, thus at 0.25 g and 0.375 g. of aspartame respectively in the 2 categories of countries mentioned above. These two estimates of average daily aspartame consumption level per head of the population would correspond to 0.11 and 0.17 g. of aspartic acid respectively, say, all in all a daily intake of the order of 0.14 g. per capita consumption of aspartic acid deriving from aspartame. In Belgium, the average weight per head of the population is of the order of 50 Kg (weighted average), that of the adults being of the order of 60 Kg for both sexes. Per KG BODY WEIGHT, the daily intake of ASPARTIC ACID deriving from aspartame, per head of the population, would thus not exceed 0.003 g (0.14 : 50).

Let us consider 3 different subjects : I. a weaned infant, II. a 10 year old child, and III. the adult : what do these 3 subjects consume

A/ of aspartic acid and glutamic acids deriving from the proteins of their daily diet (see above p. 2 to 4), and

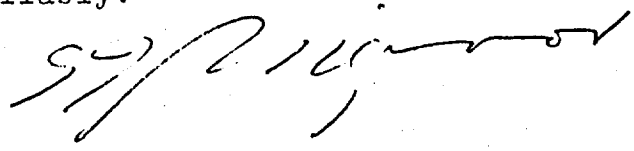
B/ of aspartic acid deriving from aspartame, at a high average level of aspartame consumption.

ADDENDUM

The validity of using an intubation technique in animals weighing only a few g. is also questionable. A dose of 0.5 ml water given by intubation to a 5 g. mouse is equivalent to 10 % of the body weight.

Even if the animal's stomach has enough capacity and mechanical strength to withstand this treatment such a dose must create an osmotic disturbance in the electrolyte and water distribution between the extra andintracellular body fluids and lead to histological changes in the tissues. Even a dose of water only one half of the volume of 0,5 ml is still an extremely large one compared to the intracellular and extracellular fluid comportments of body tissues. The significance of this disturbance is difficult to evaluate but it may be worth considering the corresponding situation of pumping several hundred ml. into a 4 Kg newly born infant in a single shot. Such an infant normally requires an intake of about 130 KCal/KG bodyweight in each 24 hr and this is generally provided by a maximum liquid food intake of approximately 150 ml/100 KCal or a total of about 700 ml not given in one dose but spread over four or five meals(See the Food and Nutrition Board Report of 1963, of the Academy of Sciences in Washington and of the National Research Council 1964. See also Consolazio's paper in World's Reviews of Nutrition and Dietetics, Vol. 4, 1963, p. 55). Rats or mice on the contrary are animals eating and drinking all day long on a continuous regime of intake. Their fluid intake proceeds therefore on a very gradual basis, reducing osmotic disturbances to a strict minimum if not to nil.

The use of the single dose intubation technique in newborn mice with its inevitably disturbing osmotic effects, must therefore be considered inappropriate as a means of toxicological evaluation, and the interpretation of the histological findings will remain a dubious exercice until more experiments have been carried out. Methods of toxicological evaluation of food additives which do not consist in incorporating the tested substance in the animals food are, in fact, unsuitable for the purpose. Their results are much more difficult to interprete reliably.



mg aspartic acid and glutamic acid per 100 g. food
and the content of protein in the sum of both, expressed in percent.
(see FAO-WHO Tables of aminoacid contents of food (Rome 1970)).

In 100 g. food	A mg. aspartic acid	B mg. glutamic acid	C A + B	D Total aminoacids	E protein (sum of aminoacid residues D x 0.85	F A + B in p.cent of E
Cows milk	264	764	1028	3553	3020	34%
Meat						
beef & veal	562	955	1517	6065	5155	29%
mutton & lamb	1373	2305	3678	15224	12940	28%
pork	1060	1718	2778	11496	9772	28%
chicken	1834	3002	4836	18026	15322	31%
Mean value for meat	-	-	-	-	-	29%
fish	1947	2655	4602	18338	15587	30%
egg	1190	1576	2766	12763	10848	25%
Mean value for animal protein	-	-	-	-	-	29.5%
Wheat flour (60-70% extraction)	374	3288	3662	9253	7865	46%
beans	2648	3271	5919	20043	17036	36%
potato	248	204	452	1572	1336	34%
Mean value for vegetable protein	-	-	-	-	-	39%

Weighted general mean value 34.8%

assuming a diet formed of
55% vegetable protein
and 45% animal protein

39 x 0.55 = 21.5
29.5 x 0.45 = 13.3

34.8

any rash judgements or extrapolations from mouse to man can be made. At present all one can say is that Dr. Olney's work appears to be an interesting research observation which is species specific.

There are apparently unpublished data of a few tests made by Olney on 10 to 20 day old mice which sustain hypothalamic lesions from tubefed aspartame, but the dosage is particularly high : 2 to 2.5 g. per Kg body weight. In a man weighing 60 Kg, this would correspond to 120 to 150 g, a quantity 300 to 600 times greater than the maximum amount of aspartame required to replace the sweetening potency of one half of the total load of the human diet in sucrose (120 to 150 : 0.25 = 500 to 600; 120 to 150 : 0.375 = 300 to 400). Tests performed with such overdosage are unrealistic and meaningless. It would be like wondering what would happen to a man consuming 300 to 600 times more sugar than the amount of it he usually consumes.

As regards the validity of his technique of tube feeding of such small animals as 10 day old mice, one would like also to see what the results of his procedure would be, when testing the safety of several additives which have been recognized as safe, for instance lactic acid and also Na and K lactates, or citric acid and its mono, di and tribasic Na and K salts, or even an additive such as sodium l. ascorbate used as antioxidant.

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- one table in appendix
- an addendum

Brain Damage in Neonatal Mice Following Monosodium Glutamate (MSG) Administration: Possible Involvement of Hypernatremia and Hyperosmolality.

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EXTRACT

We examined the effects of glutamic acid hydrochloride and of sodium chloride at levels equimolar and hyperosmolar to those used in obtaining brain damage with monosodium glutamate (MSG) in neonatal mice. At 2 mg/gm of glutamic acid hydrochloride, ten of eleven animals sustained lesions in the arcuate nucleus as well as in other brain regions. Fifteen infants receiving sodium chloride at dosages equimolar to 1, 2 and 4 mg/gm of MSG did not exhibit lesions in the arcuate or preoptic nuclei or in any other brain area. However, when 5 day old neonates were given a 4 mg/gm equimolar dose, a single animal exhibited extensive lesions in many areas of the brain (Table II) but again, none in the arcuate-preoptic area. At higher dosages of sodium chloride neuronal damage sometimes occurred in structures bordering hemorrhagic ventricular vessels. In some structures such as cerebral cortex, caudate-putamen and cerebellar cortex no obvious vessel dilatation was seen. Neuronal lesions in the cerebral cortex radiated dorsally over the corpus callosum and extended almost uniformly throughout the entire hemisphere. Pyknotic nuclei in the cerebellum extended throughout the internal granular layer of several folia, while dilatation and hemorrhage of vessels was most conspicuous in the white matter of the medullary core. Mean plasma levels of sodium rose from control values of 130.0±1.5 (S.E.M.) meq/l to 150.3±1.0 and 154.7±0.9 meq/l for MSG and sodium chloride-treated neonates, respectively. In none of the 16 mouse brains examined following administration of sucrose was there any evidence of neuronal lesions in the arcuate nucleus or any other area of the brain (Table I). All mice did,however, exhibit abnormalities in brain vasculature.

Hypernatremia is induced in neonatal mice at the highest dosage of

MSG used to elicit neuropathologic changes. Glutamic acid hydrochloride can elicit the same pattern of brain lesions in neonatal mice as can MSG at high dosages. Therefore, hypernatremia is not necessary to open the blood brain barrier for glutamate-induced neuronal lesions. Sodium chloride, although not causing arcuate lesions, is capable of causing a wide spectrum of damage in the neonatal mouse brain, at lower levels (0.02-0.05 meq/l) than previously recognized. Glutamic acid and sodium do cause brain lesions often in the same structures but their pattern of brain damage differs, probably as a result of different routes of entry into the brain.

SPECULATION

The finding that neonatal mice made hypernatremic show massive neuronal lesions due to cell dehydration in addition to brain vascular changes may explain some of the severe neurological disorders known to follow hypernatremia in infants. The fact that relatively low levels of sodium are capable of "breaking down" the blood brain barrier may give impetus to pharmacological studies involving substances which may enter the brain during mild episodes of hypernatremia. Glutamic acid hydrochloride, NaCl and sucrose are all capable of eliciting brain damage when given in sufficiently high dosages to neonatal mice. However, three different patterns of damage are encountered: lesions adjacent to cerebrospinal fluid (glutamic acid hydrochloride); focal lesions and vascular dilatation and hemorrhage (NaCl); and vascular dilatation (sucrose). These observations point up the need for considering the specific chemical moiety inducing hyperosmolality clinically.

INTRODUCTION

Monosodium glutamate (MSG) administered orally (12) or by injection (2,17) to neonatal mice results in substantial damage to the arcuate nucleus of the hypothalamus. While the arcuate nucleus is the most susceptible brain area, lesions are also commonly observed in the cerebellum, cerebral cortex, hippocampus-dentate gyrus, thalamus, tectum, habenula, subfornical organ and even olfactory bulbs (12).

At present, arcuate damage following MSG administration in the neonatal period appears to be a phenomenon restricted among mammals to rodents (2,4,11,12,17,21). The "MSG lesion" in the rodent hypothalamus is of considerable interest because of its potential use as a means of ablating the arcuate nucleus for various neuroendocrine and neuroanatomical studies (25,26). Although one laboratory (18,20) has reported hypothalamic damage in the neonatal monkey following high dosages of MSG, no other investigators have been able to confirm this finding (1,16,27,32).

To elicit brain lesions, MSG has to be administered in high dosages (2,4,11,12,17,21) which probably render the experimental animals hypernatremic. Since MSG contains both sodium and glutamic acid, lesions could result from either glutamate or sodium or both moieties acting in concert. To test these possibilities, we examined the effects of glutamic acid hydrochloride and of sodium chloride at concentrations equimolar and hyperosmolar to those of MSG known to produce brain damage in neonatal mice. Further, we studied the effects of similar concentrations of sucrose to determine if hyperosmolarity per se plays a causative role in the production of mouse brain lesions.

MATERIAL AND METHODS

1. Animal dosing.

All mice used in this study were ICR or A/JAX-ICR hybrids between 4 and 12 days of age. Compounds were administered orally via stomach tube. After treatment, mice were isolated from their dams but kept warm on a warming table until the end of the treatment interval, usually $2\frac{1}{2}$ to 5 hours.

Sodium chloride as a 10% solution (w/v) was administered at dosages equimolar for sodium to 1, 2, 4, 6, 8 and 10 mg/gm body weight of MSG. These equimolar dosages calculate as 0.31, 0.62, 1.25, 1.88, 2.50 and 3.12 mg/gm body weight of NaCl.

Glutamic acid hydrochloride was administered at 2 and 4 mg/gm body weight as a 20% solution (w/v).

Sucrose as an 80% solution (w/v) was administered at dosages equimolar to 4, 8 and 10 mg/gm body weight of MSG. These equimolar dosages calculate as 14.6, 29.2 and 36.5 mg/gm body weight of sucrose.

2. Preparation of brains for light microscopy.

Brains were fixed by immersion in either 10% or full strength formalin. Following routine dehydration and embedding, sections 6 to 8 μ thick were cut and stained with Cresyl Echt Violet solution. All brains were cut and analysed serially in the sagittal plane which included olfactory bulb to cervical spinal cord.

3. Preparation of brains for electron microscopy.

Mice were perfused with 4% glutaraldehyde and 3% paraformaldehyde in 0.1M phosphate buffer (pH 7.3) via a sharp cannula inserted into the left ventricle. Semithin (1 μ) sections stained with Methylene blue -- Azure II were examined in the sagittal plane from blocks dissected to

include diencephalon-midbrain levels. Details on the methods employed in this study can be found in a previous paper (12).

4. Sodium determinations.

In order to compare plasma sodium levels, 15 infant mice were dosed with NaCl equimolar to 4 mg/gm body weight of MSG, 36 mice were dosed with 4 mg/gm body weight of MSG and 14 mice were dosed with distilled water to serve as controls. All infants were etherized 15 minutes after dosing, the chest opened and blood withdrawn from the beating left ventricle with a heparinized capillary tube. Plasma samples from two or three mice were pooled to insure adequate volume for sodium determination by autoanalyzer.

RESULTS

Data were obtained both from serial paraffin sections and 1μ plastic (epon) sections. In analyzing paraffin sections for saline or sucrose lesions, the same criteria were used as those established for MSG damage: an area showing abundant pyknotic nuclei accompanied by edema of the neuropil. The word "lesion" is used to specify brain damage involving neurons and the neuropil as being distinct from brain damage involving changes in the blood vasculature. Careful use of control sections was necessary to avoid misinterpretations especially in young brains. Epon sections through the diencephalon-midbrain and cerebellum were used for assessing glutamic acid hydrochloride effects.

GLUTAMIC ACID HYDROCHLORIDE

Glutamic acid hydrochloride at a level of 4 mg/gm was found to be lethal to most animals. Dosages of 2 mg/gm also resulted in 54% lethality. At 2 mg/gm, ten of eleven animals sustained lesions in the arcuate nucleus (Table I). Other susceptible regions included preoptic nucleus, pretectal area, tectum, subcommissural organ, subfornical organ (Fig. 1) and cerebellum (Fig. 2a,b). The arcuate nucleus from one animal examined with the electron microscope exhibited neuronal and neuropil damage similar to that found after administration of MSG.

SODIUM CHLORIDE

Fifteen infants (7-9 days old) receiving sodium chloride at dosages equimolar to 1, 2 and 4 mg/gm of MSG did not exhibit lesions in the arcuate or preoptic nuclei or in any other brain area. However, when 5 day old neonates were given a 4 mg/gm equimolar dose, a single animal exhibited extensive lesions in many areas of the brain (Table II) but

again none in the arcuate-preoptic area. Lesions in this neonate consisted of pyknotic nuclei which in most but not all instances were coupled with edematous neuropil. Damaged cells occurred as foci or bands within cerebral cortex, caudate-putamen, cerebellum, habenula, dentate-hippocampus and thalamus. Since this severe damage was observed only in a five day old mouse, further experiments using higher dosages of sodium chloride were performed on animals 3 to 6 days old.

At 6 mg equivalency, three out of six animals sustained neuronal lesions (Table I). At 8 and 10 mg equivalencies, the incidence of animals with lesions increased as did the number of structures per brain which contained lesions. Susceptible brain areas, however, were generally consistent with those seen in the single 4 mg equivalency animal (Table II). The major new structures lesioned at 8 and 10 equivalencies were tectum and amygdala. Midbrain and olfactory bulb lesions were each observed in only one animal at the 10 mg equivalent dosage. Figures 5 through 9 taken from animals at all dose levels illustrate some of the affected brain areas.

Following oral administration of sodium chloride, one of the most conspicuous changes in the brain was related to the blood vasculature (Table I). During preparation of brains for immersion fixation, the presence of gross subdural hemorrhage was noted in many animals, particularly those at the higher doses. At the microscopic level, dilated and oftimes hemorrhagic vessels could be seen in the subarachnoid space surrounding cerebral cortex, tectum and cerebellum. The lateral and third ventricles were vastly enlarged and filled with distended choroid plexus as well as hemorrhagic vessels. The latter was especially true of the thalamic portion of the third ventricle.

Neuronal damage sometimes occurred in structures bordering hemorrhagic ventricular vessels. For example, habenular nucleus, dentate-hippocampus, pretectal area and thalamus, all of which lie close to the third ventricle, appeared to be damaged by rupture of choroidal veins and the greater cerebral vein (Fig. 9). Dilated and hemorrhagic blood vessels occurred within these brain regions as well (Figs. 10, 11). In some structures such as cerebral and cerebellar cortex and caudate-putamen no obvious vessel dilatation was seen. Lesions of cerebral cortex (Fig. 5) radiated dorsally over the corpus callosum, extending almost uniformly throughout the entire hemisphere. Pyknotic nuclei in the cerebellum (Fig. 7) occurred throughout the internal granular layer of several folia, while dilatation and hemorrhage of vessels was most conspicuous in the white matter of the medullary core. Caudate-putamen lesions (Fig. 6) most often occurred as internal foci but were sometimes found bordering the ventricle. In only one instance was a lesion found unaccompanied by observable vessel enlargement, choroid plexus dilatation or ventricular hemorrhage. In this case the caudate-putamen contained a region of pyknotic cells abutting the lateral ventricles.

On the other hand, animals without neuronal lesions often showed changes in the appearance of their blood vasculature. Two mice given 4 mg/gm equivalency of sodium chloride showed dilated subarachnoid vessels (Fig. 12) as well as hemorrhage in the fourth ventricle. The blood vasculature of all other animals given 1, 2 or 4 mg/gm of sodium chloride equivalents was normal. In contrast, all animals given 6, 8 and 10 equivalencies of sodium chloride showed pathological changes in brain vasculature. At 6, changes were limited to enlarged vessels within brain tissue, while at 8 and 10, in addition to enlarged vessels, the most prominent

change was dilatation of the subarachnoid vessels over cerebellum, tectum and cerebral cortex and of vessels within the choroid plexus.

SODIUM LEVELS

Mean plasma levels of sodium rose from control values of 130.0±1.5 (S.E.M.) meq/l to 150.3±1.0 and 154.7±0.9 meq/l for MSG and sodium chloride-treated neonates, respectively. Thus, high doses (4 mg/gm) of MSG do elevate sodium levels to an extent almost equal to that following administration of equimolar levels of NaCl.

SUCROSE

Sucrose equimolar to 4, 8 and 10 mg/gm of MSG was administered orally to neonatal mice. In none of the 16 mouse brains examined was there any evidence of lesions in the arcuate nucleus or any other area of the brain (Table I). All mice did, however, exhibit abnormalities in brain vasculature. At 4 equivalency, moderately dilated vessels were seen only in cerebral cortex and cerebellum. At 8 equivalency, vessels in almost every area of the brain were moderately dilated (Fig. 3) as were those in the subarachnoid over tectum and cerebellum and in the choroid plexus. At 10 equivalency, massively dilated vessels were apparent throughout the brain and choroid plexus, with the cerebellum showing the most severe instances. Subarachnoid vessels were extremely dilated over cerebral cortex, tectum (Fig. 4) and cerebellum. At no dosage was actual hemorrhage observed. Most animals receiving the 10 equivalency dose of sucrose died within five hours.

DISCUSSION

Our findings following administration of 2 mg/gm of glutamic acid hydrochloride to infant mice confirm a previous report (19) of an arcuate nuclear lesion and extend the observations to other damaged areas which lie in close proximity to cerebrospinal fluid (CSF). These results lend further credence to our hypothesis that MSG damage to brain structures is mediated by CSF (12).

Oral administration of sodium chloride was performed at equimolar concentration to the sodium in MSG in order to establish whether the glutamate or the sodium moiety or both acting in concert were responsible for arcuate nuclear damage. In no instances did sodium chloride administered at 1, 2, 4, 6, 8 or 10 mg/gm equivalencies cause arcuate damage, but damage to other brain regions did occur. Administering sodium at the higher dosages (4 to 10 mg/gm body weight equivalencies) revealed that almost without exception "sodium lesions" were correlated with blood vessel dilatation and/or hemorrhage.

Hypernatremia is of grave clinical concern. There are numerous reports of high serum sodium levels in infants arising from dehydration, anorexia or accidental salt poisoning (7,22). Mass salt poisoning, in which six infants died, occurred in a newborn nursery when salt was accidentally substituted for sucrose during formula preparation (8). Maternal death following the infusion of hypertonic saline into the amniotic sac to induce abortion became so prevalent in Japan (196.6 per 100,000 cases) that the technique was abandoned by the Japanese in 1950 (31). Recently, three deaths resulting from intra-amniotic instillation of saline were reported in England (5). Neuropathological descriptions of the infant (8) and adult brains (5) indicated widespread vascular damage including

capillary and venous congestion and subarachnoid hemorrhages. Neuronal necrosis of numerous regions including fatal acute hemorrhagic infarction of the amygdaloid nuclei and of the pons were reported in the adult brains. Hypernatremia has been induced in experimental animals usually by the injection or infusion of hypertonic saline solutions. Marked subdural hemorrhages were found in kittens (9,13) and petechial hemorrhages in cerebrum and cerebellum were characteristic of rabbit brains (28). Thus, there are marked similarities in the nature of the vascular damage resulting from hypernatremia in human adults and infants and in experimental animals.

Most striking in the current experiments is the fact that dosages of sodium chloride eliciting brain damage in neonatal mice were lower than those levels reported from other experimental studies or those determined after accidental salt poisonings. The mice receiving sodium chloride equivalent to 4 to 10 mg/gm of MSG were deriving 0.02 to 0.05 meq/gm of sodium. The kittens were given 0.525 meq/gm (9); the rabbits (28) received 0.06 to 0.17 meq/gm total dose. It should be noted further that saline was infused or injected in these experiments, in comparison to the infant mice dosed by stomach tube where movement of ions into the bloodstream can be expected to be somewhat slower. Data as to the amounts of salt-containing formula consumed are not available from the mass nursery poisoning (8). However, another case report (7) of accidental salt poisoning permits calculation of sodium intake as between 0.11 and 0.15 meq/gm. This infant survived but with severe brain damage.

Determination of plasma sodium levels in neonatal mice treated with MSG or equimolar sodium has established that hypernatremia does indeed result from the oral loads given. However, the extent of hypernatremia in the neonatal mice, an increase of 20 to 25 meq/l of plasma sodium, is

relatively modest. Serum sodium levels for the kittens averaged 196 (9) and those for the rabbits, approximately 200 meq/l (28). Amazingly, one infant who displayed acute neurologic symptoms derived from salt poisoning yet survived was found to have a plasma sodium level of 274, others ranged from 162 to 205 meq/l (7,8).

Our observations in neonatal mice add to the spectrum of brain vasculature damage already described. At sodium levels ranging from 0.02 to 0.05 meq/l, internal hemorrhaging was found not only in the cerebellar white matter but also in such structures as fornix, choroid plexus, tectum, thalamus and spinal cord. Massive hemorrhage of vessels in the thalamic portion of the third ventricle was also noted. The distention and breakage of subarachnoid and large intracranial blood vessels has usually been ascribed to a reduction of cerebrospinal fluid pressure combined with brain shrinkage (10).

Conspicuously lacking, however, has been actual assessment of the brain for specific areas of neuronal damage resulting from dessication. Such knowledge might explain some of the behavioral symptoms and irreversible brain damage described in cases of hypernatremia whether caused by salt poisoning or dehydration. In the neonatal mouse, the most frequently and extensively damaged areas were the caudate-putamen, cerebral cortex and cerebellum (Table II). At the highest dosages, these structures were entirely filled with pyknotic neurons and edematous neuropil. Had these animals been allowed to survive, extensive loss of function controlled by these areas would be anticipated. Thus symptoms such as tremor, ataxia, changes in the EEG and possibly convulsions observed in the poisoned human infants (7,8) and in animal experiments (9,28) might be partially explained. Previously reported symptoms such as lethargy, vomiting and

hyperirritability (7,8) are less easily related to those areas of mouse brain containing massive neuronal lesions.

Hyperosmolarity can alter the blood brain barrier to permit entry of a molecule as large as trypan blue into the brain in adult rat (6) and cat (23). It has been postulated that sodium chloride, urea and sucrose at hypertonic levels "break down" the blood brain barrier (24) by causing endothelial cell shrinkage with the resultant opening of tight junctions (30). This process has been visualized with the electron microscope after infusion of hyperosmolar urea into rabbits (3). Interestingly, the blood brain barrier in all areas of cerebral cortex was not found to be equally susceptible to disruption by hyperosmolal solutions. It thus seems likely that damage to the brains of the neonatal mice in the present study is of at least two derivations. First, areas of mouse brain exhibiting pyknosis and edema in the absence of hemorrhagic vessels (caudate-putamen, cerebellar cortex, cerebral cortex) probably reflect a localized "opening" of capillaries, arterioles or venules at the endothelial level. Sodium entering the intercellular spaces results in neuronal dessication and cell death since it does not readily enter neurons (30). Other areas of mouse brain, however, exhibit damage more likely attributable to anoxia-ischemia. For example, the thalamus contained hemorrhages throughout along with general tissue necrosis at the highest saline dosages. Further, thalamic, habenular, pretectal and hippocampal lesions appeared most severe when ruptured vessels were found in the thalamic portion of the third ventricle.

Generalized brain shrinkage occurred in the mice receiving sucrose but pyknosis and edema were not observed at the cellular level. These results probably reflect the fact that hyperosmotic glucose, like sodium,

opens the blood brain barrier (30) and penetrates into the neuropil. However, unlike sodium, glucose enters neurons more readily and thus does not cause such severe neuronal dehydration. That a certain degree of cellular dehydration does occur is evident from the enlarged blood vessels found in all animals receiving hyperosmolar dosages of sucrose. Neurologic dysfunction associated with hyperglycemia is well-known (14) and has recently been reported for hyperosmolality following galactose ingestion as well (15). Recently, accidental glucose poisoning of a 6 year old child was reported (29). Severe brain damage ensued which was attributed to anoxia, acidosis and hyperthermia, sequelae of the hyperosmolality. Few infant mice survived the higher sucrose dosages. Because neither vascular hemorrhage nor marked neuronal damage was observed, death may have resulted from metabolic disorders accompanying hypertonicity such as hypotension and acidosis.

CONCLUSIONS

While hypernatremia is induced in neonatal mice at the highest dosage of MSG used to elicit neuropathologic changes, glutamic acid hydrochloride elicits the same pattern of brain lesions as does MSG. Therefore, hypernatremia is not necessary to open the blood brain barrier for glutamate-induced neuronal lesions. Sodium chloride, although not causing arcuate nucleus lesions, is capable of causing a wide spectrum of damage in the neonatal mouse brain, at lower levels (0.02-0.05 meq/l) than previously recognized. Glutamic acid and sodium cause brain lesions often in the same structures but their pattern of brain damage differs, probably as a result of different routes of entry into the brain. Glutamate, or more probably one of its metabolites, damages cells close to circulating cerebrospinal fluid, while hypertonic saline egresses into brain tissue from capillaries, arterioles and venules. Neuronal damage following MSG administration tends to radiate inward within structures in contact with circulating CSF. Lesions following sodium chloride ingestion present themselves as foci or bands occurring throughout a given structure. Sometimes neuronal necrosis accompanies hemorrhagic vessels within a structure, a phenomenon not seen following MSG administration.

A high rate of animal mortality followed hyperosmolality induced by sucrose loads. Brain shrinkage and extensive vascular dilatation, unaccompanied by marked neuronal dehydration were the major neuropathological observations.

Finally, while neither hyperosmolarity nor hypernatremia is capable of eliciting the patterns of lesions correlated with MSG ingestion, either condition can result in severe vascular changes in the neonatal mouse brain.

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TABLE I

Compounds Administered

Dosage	Age in Days	Ratio Animals Showing Lesions* to Animals Treated	Ratio Animals Showing Vascular Abnormalities to Animals Treated
Glutamic Acid Hydrochloride			
2 mg/gm body weight	7-9	10/11	---
Sodium Chloride (equimolar dosage to mg/gm MSG)			
1	6	0/4	0/4
2	6	0/4	0/4
4	5-9	1/12	3/12
6	3-5	3/6	5/6
8	3-5	5/13	13/13
10	5-6	9/13	13/13
Sucrose (equimolar dosage to mg/gm MSG)			
4	8-12	0/10	10/10
8	6	0/5	5/5
10	7	0/1**	1/1

* Neuronal damage only.
** 5 animals were dosed at this level; only one survived the five hour interval prior to fixation.

TABLE II

Neonatal Mice with Lesions Following NaCl Treatment

Brain Structure	Dosage equimolar to mg/gm body weight of MSG 4	6	8	10
Hypothalamus (arcuate, preoptic nuclei)	0/12*	0/6	0/13	0/13
Caudate-putamen	1/12	3/6	4/13	9/13
Cerebellum	1/12	1/6	5/13	9/13
Cerebral cortex	1/12	1/6	3/13	8/13
Habenula	1/12	---	3/13	6/13
Hippocampus-dentate	1/12	---	2/13	3/13
Thalamus	1/12	---	1/13	3/13
Tectum	---	1/6	1/13	3/13
Amygdala	---	---	1/13	2/13
Olfactory bulb	---	---	---	1/13
Midbrain	---	---	---	1/13

* Figures given represent the number of animals with lesions in relation to the total number dosed.

LEGENDS

Figure 1. Sagittal section of diencephalon-midbrain of mouse receiving 2 mg/gm of glutamic acid hydrochloride. Damage occurred in arcuate nucleus (AR), preoptic nucleus (PO), subcommissural organ (SCO), pretectal area (PA) and tectum (TE). 20X

Figure 2. a. Lesions in cerebellar folia after administration of 2 mg/gm of glutamic acid hydrochloride. 88X

b. Higher magnification of a portion of the cerebellar folium showing swollen Purkinje cells and edematous neuropil. 280X

Figure 3. Dilatation of blood vessels in medullary core of cerebellum after administration of 8 equivalencies of sucrose. 75X

Figure 4. Massive dilatation of subarachnoid vessels over tectum after 10 equivalencies of sucrose. 60X

Figure 5. Cerebral cortex showing neuronal pyknosis and edema of neuropil from mouse given 8 equivalencies of sodium chloride. 60X

Figure 6. Pyknotic neurons almost entirely fill the caudate-putamen of mouse receiving 10 equivalencies of sodium chloride. 56X

Figure 7. Extensive neuronal pyknosis of internal granular layer of cerebellar folia of mouse receiving 10 equivalencies of sodium chloride. 94X

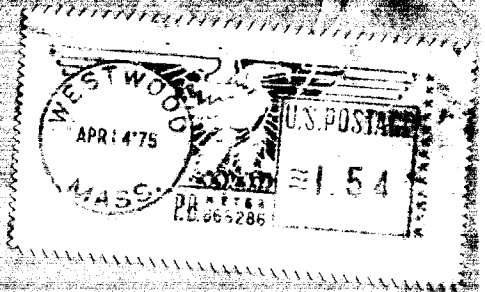
Figure 8. Neuronal degeneration and edema of neuropil in thalamus of mouse receiving 4 equivalencies of sodium chloride. 70X

Figure 9. Anoxic-ischemic changes in habenula (HA), pretectal area (PA), thalamus (T) and hippocampus (HI) around a portion of the third ventricle containing hemorrhagic blood vessels and dilated choroid plexus. 90X

Figure 10. Portion of the thalamus from an animal receiving 8 equivalencies of sodium chloride. Widespread damage including hemorrhage (arrows) has occurred. 80X

Figure 11. Higher magnification of the hemorrhage seen in Fig. 10. 160X

Figure 12. Congested and dilated subarachnoid vessels over tectum of neonatal mouse receiving 4 equivalencies of sodium chloride. This mouse had no neuronal lesions. 70X



CLASS

FIRST CLASS

INTERNATIONAL GLUTAMATE
TECHNICAL COMMITTEE
P. O. BOX 744
WESTWOOD, MASSACHUSETTS 02090

To:

Ms. Jennie Peterson
Hearing Clerk
FDA Room 4-65
Parklawn Building
5600 Fischers Lane
Rockville, MD 20852

FIRST CLASS MAIL

March 11, 1975

George W. Irving, Ph.D.
Research Associate
Life Sciences Research Office
Federation of American Societies for
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9650 Rockville Pike
Bethesda, Maryland 20014

Dear Dr. Irving:

Please recall that in your letter of November 5, 1974, you requested further data on research on glutamate in several areas. We provided replies to a number of these questions in our letter to you of December 12, 1974, at which time we submitted considerable additional data.

In the interval, since our December mailing, we have obtained additional information from work at the Universities of Iowa and Illinois. Thus, I wish to bring to the attention of the Committee the attached report and two pre-prints on glutamate.

Recall that the question had been raised as to what effect administration of MSG would have on levels of circulating amino acids, the so-called possibility of a "pulsing" effect. As a result, we asked Dr. Stegink to run complete aminograms on blood samples from women who had received MSG either in water or in Slender ®. Recall that an initial report of this study appeared in the Proceedings of the Society for Experimental Biology and Medicine (140 836 (1972)) and had been previously submitted to you. The complete aminograms have now been forwarded to us and copies are attached for SCOGS review.

In addition, your letter of November 5th, asked questions as to metabolism of MSG and also referred to the placental transfer of glutamate. This has been studied by the groups at Illinois and Iowa, and we attach pre-prints of a metabolism paper which is in press at the American Journal of Physiology and a placental transfer paper which is in press at the American Journal of Obstetrics and Gynecology.

Copies of these data are being sent to the Hearing Clerk at F.D.A. although we request that any mention of this work prior to publication in the literature carry proper identification as a courtesy to the scientists who have completed this research.

We hope the SCOGS finds this data of interest.

Sincerely,

A. G. Ebert C.L.P.

Chairman

Andrew G. Ebert/map1/19

cc: FDA, Hearing Clerk

MONOSODIUM GLUTAMATE: EFFECT ON PLASMA AND BREAST MILK AMINO ACID LEVELS IN LACTATING WOMEN

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The project described was published in the Proc. Soc. Exptl. Biol & Med., 140, 836-841 (1972).

TABLE I

PLASMA AMINO ACID LEVELS IN LACTATING WOMEN AFTER GLUTAMATE LOADING (μmoles/dl)

PLASMA AMINO ACID LEVELS

TIME (min)	TAURINE	THREONINE	SERINE	PROLINE	CITRULLINE	GLYCINE
			μmoles/100 ml			
MSG WITH WATER (n=4)						
0	5.10 ± 1.16	13.9 ± 3.43	17.7 ± 7.87	40.2 ± 25.5	4.07 ± 0.85	28.6 ± 2.62
30	4.96 ± 0.28	14.6 ± 3.20	20.8 ± 3.71	36.0 ± 24.0	3.59 ± 1.06	39.6 ± 11.1
60	5.51 ± 1.15	13.0 ± 3.12	15.7 ± 0.78	31.8 ± 18.7	3.12 ± 0.38	39.8 ± 20.1
120	4.07 ± 0.84	12.3 ± 3.81	17.3 ± 2.66	31.6 ± 12.8	2.93 ± 0.61	38.9 ± 16.1
180	6.91 ± 1.75	11.7 ± 3.81	18.5 ± 3.76	29.6 ± 12.8	2.73 ± 0.80	30.1 ± 3.78
MSG WITH SLENDER (n=9)						
0	6.27 ± 2.26	17.3 ± 1.75	20.1 ± 6.95	30.1 ± 13.5	4.61 ± 1.43	36.6 ± 12.7
60	6.07 ± 3.22	21.1 ± 7.51	31.1 ± 7.48	53.1 ± 22.9	4.18 ± 1.00	38.6 ± 10.1
90	5.31 ± 2.56	23.0 ± 4.91	26.0 ± 5.46	53.6 ± 20.1	3.98 ± 0.79	38.8 ± 10.3
150	6.15 ± 2.88	19.5 ± 5.25	21.9 ± 5.25	35.2 ± 6.99	4.01 ± 0.87	32.9 ± 14.3
210	7.40 ± 2.29	16.2 ± 2.51	20.1 ± 2.81	37.3 ± 16.6	3.96 ± 0.67	32.7 ± 9.55
LACTOSE WITH WATER (n=4)						
0	2.85 ± 1.80	14.8 ± 1.58	20.9 ± 5.67	18.0 ± 6.3	4.03 ± 0.45	25.2 ± 2.81
30	3.28 ± 0.90	17.5 ± 0.85	18.7 ± 3.05	26.3 ± 11.1	3.79 ± 1.00	26.4 ± 30.0
60	2.00 ± 0.45	19.8 ± 5.23	19.7 ± 5.03	28.3 ± 4.75	4.38 ± 1.09	38.4 ± 6.13
90	2.45 ± 1.00	17.2 ± 7.94	18.0 ± 3.51	31.5 ± 3.73	3.27 ± 1.74	24.6 ± 11.5
150	3.82 ± 2.10	12.5 ± 7.41	13.6 ± 8.34	27.4 ± 8.51	3.11 ± 1.46	19.2 ± 7.12
210	3.89 ± 0.11	15.9 ± 2.14	18.7 ± 2.70	24.7 ± 2.86	4.01 ± 0.97	24.5 ± 4.88

PLASMA FREE AMINO ACID LEVELS

TIME (min)	ASPARTATE	GLUTAMINE	GLUTAMATE	ALANINE
MSG WITH WATER (n=4)		µmoles/100 ml		
0	0.32 ± 0.16	61.9 ± 16.8	3.90 ± 1.70	53.1 ± 13.3
30	1.04 ± 0.92	68.0 ± 7.10	13.0 ± 10.1	50.6 ± 11.4
60	0.54 ± 0.31	62.4 ± 5.80	7.50 ± 4.80	46.6 ± 5.60
120	0.70 ± 0.40	65.9 ± 14.9	5.10 ± 2.70	47.3 ± 10.5
180	0.45 ± 0.25	62.4 ± 8.07	4.75 ± 2.31	40.5 ± 10.5
MSG WITH SLENDER (n=9)				
0	0.64 ± 0.27	61.0 ± 3.70	4.34 ± 0.70	42.5 ± 6.32
60	1.28 ± 1.42	70.5 ± 14.7	7.05 ± 2.70	68.1 ± 19.6
90	1.27 ± 1.24	72.1 ± 14.0	9.23 ± 5.34	57.3 ± 12.1
150	1.84 ± 1.45	65.0 ± 10.4	11.8 ± 8.20	50.3 ± 10.4
210	1.32 ± 0.80	59.6 ± 8.31	10.2 ± 7.99	46.8 ± 6.95
LACTOSE WITH WATER (n=4)				
0	0.43 ± 0.14	69.7 ± 16.6	4.00 ± 0.97	40.5 ± 6.60
30	0.65 ± 0.07	83.6 ± 8.70	4.10 ± 0.28	48.3 ± 6.70
60	0.39 ± 0.10	76.4 ± 4.60	3.70 ± 1.40	46.3 ± 10.0
90	0.56 ± 0.26	65.0 ± 16.6	3.80 ± 1.90	41.7 ± 11.4
150	0.61 ± 0.26	71.2 ± 10.9	5.70 ± 4.40	35.1 ± 3.50
210	0.26 ± 0.10	63.0 ± 14.1	3.20 ± 0.56	35.9 ± 4.00

PLASMA AMINO ACID LEVELS

Time (min.)	α-AMINOBUTYRATE	VALINE	½-CYSTINE	METHIONINE	ISOLEUCINE	LEUCINE
MSG WITH WATER (n=4)			μmoles/100 ml			
0	1.83 ± 0.95	20.1 ± 4.55	7.52 ± 1.95	1.86 ± 0.77	4.32 ± 1.29	6.99 ± 3.17
30	2.73 ± 0.96	17.9 ± 1.61	8.92 ± 1.47	2.29 ± 0.29	4.45 ± 0.33	6.69 ± 1.57
60	2.06 ± 0.66	14.4 ± 1.60	8.48 ± 1.47	1.70 ± 0.47	3.41 ± 0.67	5.84 ± 1.29
120	2.15 ± 0.78	16.8 ± 1.91	8.57 ± 1.65	1.91 ± 0.31	4.01 ± 0.32	5.98 ± 1.25
180	2.94 ± 0.81	20.1 ± 3.81	8.61 ± 1.71	1.55 ± 0.38	4.15 ± 0.67	5.17 ± 1.75
MSG WITH SLENDER (n=9)						
0	2.87 ± 0.76	22.4 ± 3.81	8.68 ± 1.79	2.81 ± 0.36	6.42 ± 0.36	12.8 ± 2.41
60	3.43 ± 0.80	34.0 ± 8.31	10.3 ± 1.98	5.40 ± 1.55	12.1 ± 3.95	24.8 ± 7.10
90	3.28 ± 0.75	34.0 ± 6.20	9.50 ± 2.44	5.23 ± 0.83	11.4 ± 3.36	22.7 ± 4.08
150	2.56 ± 0.49	29.0 ± 4.41	8.88 ± 3.33	3.90 ± 0.48	7.37 ± 1.85	16.4 ± 3.17
210	2.62 ± 0.59	25.5 ± 3.32	7.43 ± 1.50	2.51 ± 0.49	5.60 ± 0.67	12.8 ± 0.81
LACTOSE WITH WATER (n=4)						
0	1.85 ± 0.36	22.3 ± 1.19	7.53 ± 1.57	2.33 ± 0.12	6.10 ± 0.77	12.5 ± 1.23
30	2.13 ± 0.27	28.8 ± 6.81	8.33 ± 2.24	3.18 ± 1.31	9.00 ± 2.31	15.1 ± 5.15
60	1.48 ± 0.24	32.4 ± 2.86	7.98 ± 3.31	3.80 ± 1.40	9.82 ± 3.41	21.7 ± 6.58
90	1.73 ± 0.62	25.5 ± 13.4	7.99 ± 2.29	3.48 ± 1.74	7.75 ± 3.29	17.6 ± 8.11
150	1.25 ± 0.71	17.9 ± 10.1	8.55 ± 2.97	2.05 ± 1.01	4.89 ± 2.59	12.1 ± 5.06
210	2.03 ± 0.81	29.4 ± 3.55	7.84 ± 2.10	2.61 ± 0.20	6.49 ± 6.06	14.1 ± 1.27

PLASMA AMINO ACID LEVELS

TIME (min)	TYROSINE	PHENYLALANINE	ORNITHINE	LYSINE	HISTIDINE	ARGININE
MSG WITH WATER (n=4)			µmoles/100 ml			
0	6.24 ± 2.30	5.72 ± 1.68	11.7 ± 2.02	18.6 ± 2.76	8.49 ± 1.91	4.71 ± 1.57
30	6.69 ± 1.25	6.09 ± 1.14	12.2 ± 1.79	20.3 ± 1.40	8.91 ± 0.69	6.29 ± 1.96
60	5.84 ± 2.09	5.69 ± 1.38	12.7 ± 1.09	17.1 ± 0.79	7.51 ± 1.19	6.24 ± 1.14
120	5.98 ± 0.89	5.55 ± 0.85	11.2 ± 1.11	18.6 ± 1.28	7.78 ± 0.27	5.83 ± 2.07
180	5.17 ± 0.61	5.00 ± 0.72	11.3 ± 2.75	18.4 ± 0.99	7.19 ± 1.15	5.08 ± 1.15
MSG WITH SLENDER (n=9)						
0	6.99 ± 1.51	6.57 ± 1.18	12.8 ± 3.00	23.2 ± 4.01	8.61 ± 0.83	7.24 ± 1.72
60	13.9 ± 4.40	10.4 ± 3.24	15.9 ± 3.63	37.1 ± 10.3	11.0 ± 1.44	12.4 ± 3.81
90	13.8 ± 1.73	10.1 ± 1.81	16.5 ± 4.20	37.0 ± 7.86	10.5 ± 1.29	12.2 ± 2.94
150	10.5 ± 1.96	7.36 ± 0.79	14.7 ± 3.83	27.6 ± 7.30	8.45 ± 1.37	8.75 ± 2.90
210	8.99 ± 1.01	6.59 ± 1.31	15.4 ± 3.74	22.7 ± 4.41	8.40 ± 1.33	6.98 ± 1.49
LACTOSE WITH WATER (n=4)						
0	6.13 ± 1.81	5.76 ± 0.42	11.9 ± 0.75	25.4 ± 9.30	9.98 ± 2.60	6.58 ± 2.17
30	8.30 ± 2.99	6.71 ± 2.18	12.1 ± 1.19	26.3 ± 1.84	9.31 ± 1.00	6.39 ± 1.82
60	11.8 ± 2.57	8.86 ± 1.69	15.3 ± 3.99	35.0 ± 12.8	12.9 ± 4.51	10.6 ± 3.90
90	10.0 ± 3.30	7.35 ± 3.51	14.9 ± 1.40	40.1 ± 10.0	13.2 ± 4.55	10.5 ± 6.15
150	6.44 ± 3.71	5.73 ± 2.31	13.2 ± 1.01	36.8 ± 5.92	9.85 ± 1.11	6.74 ± 4.51
210	6.87 ± 1.44	6.01 ± 0.48	12.7 ± 1.31	26.1 ± 7.77	9.49 ± 1.17	7.27 ± 3.91

TABLE 2

BREAST MILK FREE AMION ACID LEVELS IN LACTATING WOMEN AFTER GLUTAMATE LOADING
µmoles/100 ml

BREAST MILK FREE AMINO ACID LEVELS IN LACTATING WOMEN (μmoles/100 ml milk)

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
CYSTEIC ACID			
0	1.80 ± 1.02	2.26 ± 1.93	2.24 ± 1.38
1	2.12 ± 1.61	3.12 ± 2.18	2.55 ± 2.39
2	2.08 ± 1.28	3.38 ± 1.00	1.76 ± 1.22
3	1.92 ± 1.30	2.50 ± 1.50	2.09 ± 1.57
4	1.92 ± 1.04	3.03 ± 1.72	1.96 ± 1.34
6	2.65 ± 2.32	1.61 ± 0.73	1.69 ± 1.57
12	1.33 ± 1.37	1.73 ± 0.91	0.70 ± 0.35
TAURINE			
0	35.7 ± 17.8	29.8 ± 9.40	26.8 2.68 ± 8.63
1	49.3 ± 20.9	28.9 ± 13.1	31.4 ± 11.7
2	42.8 ± 13.6	36.6 ± 14.6	25.7 ± 10.4
3	37.3 ± 13.8	33.8 ± 10.6	27.8 ± 6.67
4	40.0 ± 21.1	32.2 ± 12.6	28.6 ± 13.1
6	40.9 ± 10.3	33.9 ± 12.3	28.9 ± 7.44
12	48.9 ± 17.7	33.7 ± 11.0	28.4 ± 16.1
ASPARTATE			
0	2.96 ± 1.15	2.91 ± 1.11	2.93 ± 0.90
1	3.01 ± 0.50	2.73 ± 1.48	4.99 ± 2.90
2	4.02 ± 1.63	5.29 ± 2.24	8.59 ± 2.81
3	3.93 ± 0.49	8.59 ± 4.18	7.94 ± 2.75
4	4.70 ± 4.37	8.29 ± 4.74	8.72 ± 5.27
6	4.33 ± 1.98	10.6 ± 1.51	8.61 ± 3.01
12	4.85 ± 2.05	5.99 ± 2.98	8.42 ± 2.07
THREONINE			
0	8.71 ± 2.66	9.65 ± 3.16	5.72 ± 2.24
1	10.7 ± 2.67	7.89 ± 2.75	6.15 ± 0.80
2	10.0 ± 2.56	10.9 ± 4.22	6.52 ± 1.10
3	9.82 ± 2.49	11.3 ± 3.33	6.30 ± 1.20
4	10.6 ± 7.71	9.85 ± 4.16	6.49 ± 1.69
6	8.93 ± 3.69	12.5 ± 3.47	7.48 ± 1.93
12	10.4 ± 4.96	8.37 ± 3.17	9.45 ± 4.03

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
SERINE			
0	10.9 ± 4.61	14.3 ± 4.83	12.8 ± 5.30
1	14.7 ± 4.04	11.5 ± 2.04	12.1 ± 5.10
2	11.6 ± 4.31	13.5 ± 3.95	13.1 ± 5.91
3	12.1 ± 2.58	19.0 ± 8.25	11.6 ± 6.36
4	13.2 ± 5.59	14.1 ± 5.73	16.5 ± 4.60
6	13.8 ± 5.68	16.1 ± 2.21	14.8 ± 4.44
12	18.7 ± 5.09	11.0 ± 4.78	16.5 ± 11.8
GLUTAMINE			
0	79.5 ± 44.5	51.8 ± 27.2	34.3 ± 19.1
1	79.9 ± 55.1	42.4 ± 16.2	35.8 ± 19.8
2	68.7 ± 35.5	44.5 ± 12.7	55.1 ± 20.3
3	62.6 ± 34.0	57.0 ± 19.8	52.5 ± 18.0
4	67.3 ± 37.2	53.4 ± 22.4	53.9 ± 7.7
6	70.6 ± 38.0	88.1 ± 27.5	51.0 ± 11.9
12	85.1 ± 34.1	57.9 ± 23.0	47.3 ± 12.7
GLUTAMATE			
0	128 ± 36.6	146 ± 51.1	107 ± 61.0
1	147 ± 27.1	118 ± 39.7	113 ± 27.3
2	145 ± 16.2	128 ± 50.4	126 ± 17.1
3	167 ± 51.0	150 ± 34.1	153 ± 66.7
4	175 ± 49.2	161 ± 53.8	145 ± 16.4
6	159 ± 89.5	182 ± 28.0	157 ± 35.0
12	161 ± 34.1	159 ± 32.3	181 ± 32.0
PROLINE			
0	4.30 ± 4.01	2.47 ± 1.55	4.08 ± 0.65
1	2.87 ± 1.27	2.37 ± 1.62	4.48 ± 1.13
2	2.76 ± 1.74	3.17 ± 1.22	3.69 ± 1.59
3	4.50 ± 2.75	2.84 ± 1.44	5.43 ± 3.78
4	2.58 ± 0.63	3.74 ± 1.83	2.24 ± 2.10
6	2.81 ± 1.27	2.14 ± 1.15	3.44 ± 1.99
12	4.73 ± 1.88	2.76 ± 1.42	3.14 ± 0.83

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
CITRULLINE			
0	1.33 ± 0.58	1.66 ± 0.75	1.58 ± 1.22
1	1.31 ± 0.54	1.17 ± 0.40	1.27 ± 0.84
2	1.25 ± 0.44	1.52 ± 0.74	1.64 ± 0.76
3	1.08 ± 0.40	1.47 ± 0.47	1.25 ± 0.82
4	1.19 ± 0.68	1.18 ± 0.32	1.47 ± 0.87
6	1.39 ± 0.11	2.40 ± 0.48	1.48 ± 0.81
12	1.43 ± 0.74	1.39 ± 0.61	1.80 ± 0.57
GLYCINE			
0	7.52 ± 1.98	8.89 ± 2.22	9.83 ± 5.60
1	8.48 ± 2.31	8.63 ± 3.05	11.6 ± 3.21
2	8.98 ± 3.80	9.15 ± 3.35	11.8 ± 3.82
3	10.1 ± 4.25	10.3 ± 3.77	11.7 ± 3.47
4	10.9 ± 3.88	9.83 ± 3.19	14.1 ± 4.04
6	10.3 ± 5.00	13.5 ± 2.55	11.7 ± 2.99
12	9.40 ± 5.80	9.89 ± 2.53	11.9 ± 1.96
ALANINE			
0	17.5 ± 7.41	16.0 ± 6.01	16.5 ± 6.31
1	22.7 ± 6.11	22.4 ± 4.63	22.4 ± 6.82
2	23.4 ± 5.22	30.1 ± 9.11	30.1 ± 6.63
3	23.4 ± 5.34	29.8 ± 9.81	29.8 ± 7.52
4	20.9 ± 10.9	31.0 ± 10.3	31.0 ± 5.64
6	26.1 ± 12.9	35.1 ± 7.81	35.0 ± 6.11
12	33.1 ± 10.4	35.0 ± 10.8	35.1 ± 3.69
α-AMINOBUTYRATE			
0	3.19 ± 2.54	1.44 ± 0.54	1.12 ± 1.19
1	1.45 ± 0.82	0.95 ± 0.41	1.02 ± 0.89
2	1.35 ± 0.51	1.32 ± 0.57	1.22 ± 0.66
3	1.43 ± 1.00	1.58 ± 0.68	2.75 ± 2.43
4	1.62 ± 0.92	1.63 ± 0.81	1.50 ± 1.06
6	1.59 ± 1.12	2.06 ± 0.37	1.34 ± 1.23
12	1.70 ± 0.90	1.53 ± 0.48	1.28 ± 0.50

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
VALINE			
0	5.35 ± 2.40	4.01 ± 2.05	3.39 ± 2.56
1	5.59 ± 1.83	3.24 ± 1.88	3.65 ± 1.60
2	5.15 ± 1.20	4.41 ± 2.14	3.56 ± 1.19
3	5.81 ± 1.93	5.43 ± 1.56	3.69 ± 2.01
4	6.32 ± 2.18	5.78 ± 2.92	4.04 ± 1.45
6	6.17 ± 3.98	5.99 ± 0.50	4.11 ± 1.00
12	7.40 ± 3.38	4.61 ± 1.60	4.80 ± 0.38
½-CYSTINE			
0	3.85 ± 1.62	4.43 ± 1.72	3.63 ± 1.34
1	4.61 ± 1.89	3.65 ± 0.90	4.86 ± 1.05
2	3.23 ± 1.30	3.66 ± 1.49	3.48 ± 0.58
3	3.76 ± 1.63	3.32 ± 0.95	5.22 ± 3.74
4	3.34 ± 1.91	3.18 ± 1.29	5.18 ± 1.78
6	3.33 ± 0.37	3.39 ± 1.89	4.91 ± 1.08
12	2.86 ± 1.36	2.85 ± 0.76	3.42 ± 0.39
METHIONINE			
0	0.41 ± 0.24	0.37 ± 0.30	0.29 ± 0.19
1	0.49 ± 0.02	0.44 ± 0.27	0.29 ± 0.11
2	0.55 ± 0.19	0.57 ± 0.33	0.31 ± 0.12
3	0.45 ± 0.20	0.71 ± 0.30	0.51 ± 0.17
4	0.41 ± 0.25	0.47 ± 0.18	0.31 ± 0.08
6	0.54 ± 0.51	0.46 ± 0.16	0.52 ± 0.16
12	0.58 ± 0.27	0.87 ± 0.43	0.71 ± 0.32
ISOLEUCINE			
0	0.91 ± 0.44	0.76 ± 0.44	0.61 ± 0.36
1	0.85 ± 0.25	0.77 ± 0.33	0.51 ± 0.20
2	0.78 ± 0.40	0.96 ± 0.43	0.54 ± 0.11
3	0.93 ± 0.43	0.87 ± 0.38	0.63 ± 0.17
4	1.04 ± 0.87	0.77 ± 0.34	0.78 ± 0.10
6	0.98 ± 0.51	1.11 ± 0.17	0.77 ± 0.12
12	1.07 ± 0.75	0.85 ± 0.41	0.98 ± 0.18

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
LEUCINE			
0	2.90 ± 1.14	2.40 ± 1.24	1.94 ± 1.51
1	3.04 ± 0.49	2.32 ± 1.16	1.99 ± 0.88
2	3.39 ± 0.60	3.37 ± 1.52	2.15 ± 0.61
3	3.51 ± 0.74	3.63 ± 1.16	2.32 ± 1.04
4	3.97 ± 2.27	3.18 ± 1.10	2.68 ± 1.01
6	3.85 ± 1.95	4.06 ± 0.14	2.55 ± 1.00
12	3.94 ± 1.44	2.53 ± 0.97	3.38 ± 0.68
TYROSINE			
0	0.92 ± 0.32	1.15 ± 0.63	1.64 ± 1.44
1	1.19 ± 0.32	1.21 ± 0.57	1.12 ± 0.28
2	1.38 ± 0.62	1.85 ± 0.79	1.33 ± 0.37
3	1.32 ± 0.32	2.19 ± 0.69	1.21 ± 0.42
4	1.17 ± 0.49	2.07 ± 0.80	1.13 ± 0.19
6	1.45 ± 0.90	2.30 ± 0.13	1.16 ± 0.55
12	1.29 ± 0.57	1.42 ± 0.37	1.71 ± 0.78
PHENYLALANINE			
0	1.23 ± 0.35	1.22 ± 0.56	0.90 ± 0.54
1	1.25 ± 0.19	1.04 ± 0.48	1.03 ± 0.20
2	1.41 ± 0.28	1.53 ± 0.57	1.20 ± 0.30
3	1.44 ± 0.20	1.64 ± 0.45	1.09 ± 0.27
4	1.39 ± 0.42	1.46 ± 0.55	1.04 ± 0.28
6	1.55 ± 0.42	1.60 ± 0.29	1.51 ± 0.48
12	1.79 ± 0.38	1.55 ± 0.52	1.90 ± 0.58
ETHANOLAMINE			
0	5.32 ± 2.08	6.14 ± 2.01	6.16 ± 2.58
1	4.64 ± 2.14	8.13 ± 2.96	9.30 ± 1.78
2	2.65 ± 2.60	8.55 ± 2.38	6.41 ± 2.73
3	7.22 ± 1.79	6.83 ± 3.76	4.23 ± 4.56
4	8.61 ± 2.24	6.24 ± 3.67	10.6 ± 1.93
6	4.53 ± 3.55	5.22 ± 4.28	7.44 ± 2.11
12	4.01 ± 3.75	7.95 ± 1.91	7.63 ± 3.94

TIME (HOURS)	LACTOSE WITH WATER	MSG WITH SLENDER	MSG WITH WATER
ORNITHINE			
0	1.09 ± 0.99	1.51 ± 1.06	1.57 ± 0.63
1	1.14 ± 0.83	0.95 ± 0.32	1.93 ± 0.72
2	1.25 ± 0.47	1.34 ± 0.41	1.58 ± 0.44
3	1.16 ± 0.61	1.78 ± 1.31	1.25 ± 0.16
4	1.05 ± 0.58	1.73 ± 0.81	1.58 ± 0.49
6	1.19 ± 0.40	1.92 ± 1.21	1.88 ± 0.33
12	1.30 ± 1.12	1.95 ± 1.83	0.93 ± 0.27
LYSINE			
0	1.81 ± 0.67	1.58 ± 0.52	1.35 ± 0.17
1	1.54 ± 0.14	1.35 ± 0.75	1.73 ± 0.64
2	1.81 ± 0.75	1.68 ± 0.56	1.48 ± 0.50
3	2.01 ± 0.45	1.85 ± 0.77	1.61 ± 0.80
4	1.39 ± 0.54	1.91 ± 0.71	1.90 ± 0.70
6	3.45 ± 2.75	1.97 ± 0.67	1.55 ± 0.75
12	1.81 ± 0.39	2.22 ± 0.64	2.47 ± 0.83
HISTIDINE			
0	3.77 ± 1.18	2.66 ± 0.99	2.29 ± 1.36
1	4.01 ± 1.08	2.22 ± 0.88	2.57 ± 1.03
2	2.36 ± 1.57	2.74 ± 1.14	2.48 ± 0.48
3	3.98 ± 1.15	2.86 ± 0.70	2.53 ± 0.98
4	3.80 ± 1.35	2.41 ± 0.90	2.37 ± 0.89
6	4.08 ± 2.65	2.92 ± 0.43	2.33 ± 0.79
12	3.41 ± 1.81	3.58 ± 2.09	3.81 ± 1.10
ARGININE			
0	0.94 ± 0.46	0.93 ± 0.25	0.70 ± 0.15
1	0.75 ± 0.13	0.92 ± 0.61	1.08 ± 0.59
2	0.88 ± 0.17	0.95 ± 0.41	0.86 ± 0.48
3	0.94 ± 0.23	0.97 ± 0.36	0.99 ± 0.64
4	0.99 ± 0.32	1.15 ± 0.52	1.11 ± 0.95
6	1.34 ± 0.98	1.14 ± 0.37	0.98 ± 0.77
12	0.79 ± 0.41	1.39 ± 1.15	0.82 ± 0.43

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MONOSODIUM GLUTAMATE METABOLISM IN THE NEONATAL MONKEY

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Glutamate Metabolism in the Neonatal Monkey

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Abstract:

Monosodium glutamate was administered orally to newborn monkeys at 1, 2 and 4 gm/kg body weight. Plasma glutamate and aspartate levels were rapidly elevated, in proportion to the dose administered. Other amino acid levels were not affected. Studies using ^{14}C-labeled glutamate demonstrated rapid conversion of glutamate into two major ninhydrin-negative compounds identified as glucose and lactate. It has been suggested (36) that the inability of Reynolds et al. (45) to duplicate the primate lesion originally described by Olney and Sharpe (35) was due to a failure to obtain adequate circulating levels of glutamate. These data negate this contention. Two of the neonatal monkeys studied exhibited a decreased glutamate tolerance as marked by elevated initial blood glutamate levels and an abnormal glutamate tolerance curve. Despite this handicap in handling glutamate, neither animal developed the neuronal lesion originally described by Olney and Sharpe (35).

Introduction:

It is now generally accepted that the administration of large quantities of monosodium glutamate (MSG) to the newborn mouse produces a variety of neurotoxic effects. These lesions include the retinal lesions first described by Lucas and Newhouse (22) and confirmed by other investigators (7,14,15,33,42) obesity (18,25,32,44), neuro-endocrine disturbances (32,44), possible learning defects (43), and a massive lesion in the arcuate nucleus of the hypothalamus as reported by Olney and collaborators (32,33). Neuronal necrosis has been demonstrated in the mouse following either oral (1,6,19,34) or subcutaneous doses (1,6,29,33) of MSG. Olney and Sharpe reported production of hypothalamic lesions in the infant rat, rabbit and a single immature rhesus monkey when MSG was injected subcutaneously at doses ranging from 0.5 to 2.7 gm/kg (35). Whether lesions are produced in species other than the rodent is highly uncertain at this point. Arees and Mayer (3), Burde et al. (6), and Everly (13) have produced neuronal necrosis in the rat. However,Adamo and Ratner (2) were unable to produce lesions in the rat, and Oser, et al. (37) were unable to produce lesions in either the mouse, rat or the dog. Nothing more has been written concerning the rabbit lesion since Olney and Sharpe's reference to unpublished data (35).

In 1969, Olney and Sharpe (35) reported an extensive MSG-induced neuronal lesion in a single premature primate following injection of MSG (2.7 g/kg body weight), noting that the lesion was similar to that described earlier in the mouse. Other investigators, however, failed to reproduce this lesion as originally described(1,31,45,56)with Reynolds et al.(45,46) reporting

no neuronal damage in either monkey fetus or neonate after administration of MSG from 45 to 125 days of gestation or birth to 14 days of age. Reynolds et al. (45) did observe artifactual damage similar to that described by Olney and Sharpe (35) in inadequately perfused brains from control animals. In 1972, Olney, et al. redescribed the site and size of the original lesion in the primate following oral administration of large doses of MSG (36). The lesion formerly designated as extensive and similar to the mouse lesion was now reported to involve as few as 50 to 90 cells, and to be seen only in thin plastic sections of the hypothalamus. At the present time the significance of the microlesion in the primate is unknown, unconfirmed, and awaiting further study. However, the marked difference in the size and type of lesion observed between the neurologically immature neonatal mouse and the more neurologically mature neonatal monkey is striking. Olney et al. (36) have suggested that the differences noted between their studies and those of Reynolds et al. (45) relate to the emetic properties of MSG, suggesting that the monkeys in the latter study may have vomited a substantial portion of the administered MSG dose. The data presented in this paper include plasma glutamate levels following MSG loading in the animals reported in the study of Reynolds, et al.(45) and demonstrate the enormous elevations of blood glutamate which occurred.

The failure of investigators other than Olney to detect an extensive neuronal lesion in the newborn monkey administered large oral doses of MSG (1-4 g/kg body weight) (1,31,45,56) has given rise to the proposition that a metabolite of glutamate or a decreased ability to metabolize glutamate

may be responsible for neuronal damage in the newborn mouse. In an attempt to determine what differences if any are found in glutamate metabolism between the MSG-susceptible mouse and the MSG-insensitive monkey, we have studied the effect of MSG load on plasma amino acid levels in the neonatal monkey, and determined the major metabolites of glutamate released by the liver to the circulation which might affect the brain. We have administered 3,4-^{14}C glutamate to the newborn animal by stomach tube and measured the incorporation of glutamate into both amino acids and other metabolites in the peripheral circulation. Major non-amino acid metabolites have been identified.

MATERIALS AND METHODS

The L-monosodium glutamate (International Minerals & Chemical Corp.) used assayed at greater than 99.9% glutamate by amino acid analysis. No other amino acids were noted. The 3,4-^{14}C-MSG (International Chemical and Nuclear Corp.) used had a specific activity of 200 mcuries per millimole and was diluted with unlabeled MSG as noted. Sequential blood samples were obtained from each animal at known intervals of time. All infants were closely observed after dosing. Four animals vomited small amounts (less than 1 cc) of a frothy, bile-colored fluid at 40, 55, 60 and 71 minutes after dosing (Table 1). Since considerable time had elapsed since dosing, and since the vomitus was minimal in quantity, these animals were not redosed. One infant (MP) vomited approximately 1.5 cc ten minutes after dosing and was redosed in toto; subsequent vomiting did not occur. Infant monkeys were obtained primarily from the Primate Breeding Facility, the University of Illinois at the Medical Center; two infants were purchased from

commercial sources (Table 1). Macaca speciosa and Macaca mulatta are very similar. Macaca irus is a smaller monkey at birth and in adulthood, although quite similar generically to the two larger macaque species.

Monosodium glutamate dissolved in water (50% solution, w/v) was administered by stomach tube to the infant monkeys, aged 5 hours to 17 days, at dosages indicated in Table 1. Ten animals were given glutamate, and one control animal was given water. The animals were sedated with pheneyclidin hydrochloride (Sernylan, Parke Davis) at 0.5 mg/kg intramuscularly. Following local infiltration anesthesia (procaine) a catheter was introduced into the inferior vein cava by a femoral cutdown. Cerebrospinal fluid samples were obtained by ventricular tap and prepared by the method of Dickinson and Hamilton (9). Simultaneous radioactivity and amino acid analysis was carried out by the method of Stegink (49). This technique permits detection of both ninhydrin-negative and ninhydrin-positive metabolites derived from glutamate. Plasma samples were immediately deproteinized with sulfosalicylic acid and analyzed promptly or stored at -70°. These conditions prevent conversion of glutamine to glutamate and pyrrolidone carboxylate and prevent loss of cystine (10, 39). Amino acid analyses were carried out on Technicon NC-1 amino acid analyzers using the Efron buffer system (12).

In order to identify the ninhydrin-negative metabolites of glutamate, two young monkeys (1 year) were given large loads of glutamate (1 g/kg body weight, 100 μc ^{14}C-glutamate). Blood samples were obtained at the

previously determined peak of radioactivity for the required compound. The ninhydrin-negative compounds were isolated from plasma which had been immediately deproteinized either with sulfosalicylic acid (12) or by means of a Diaflo ultrafiltration cell. Samples of deproteinized plasma were applied to an amino acid analyzer column and the eluate collected in 2.0 ml fractions. The radioactivity profile obtained in this manner was identical to that obtained using the simultaneous radioactivity amino acid analysis technique. Fractional collections were then desalted by high voltage electrophoresis (55). This electrophoretic method gives excellent separation of ninhydrin-negative acidic metabolites.

RESULTS

The administration of large oral doses of MSG (1 to 4 g/kg body weight) to the neonatal monkey resulted in the prompt and extensive elevation of plasma glutamate and aspartate levels (Figure 1). The elevations in plasma glutamate were roughly in proportion to the dose administered; this proportionality was less marked for plasma aspartate levels. Plasma levels of other amino acids, including those which potentially could be derived from glutamate or aspartate, were essentially unchanged. Plasma glutamate levels increased 40 to 80 fold from fasting levels (5 umoles/dl) reaching maximal levels 1 to 2 hours after administration, while aspartate levels increased 50 to 90 fold over baseline levels (0.4 umole/dl).

Despite marked elevations in plasma glutamate and aspartate levels, no evidence of a massive hypothalamic lesion was noted in any of these animals as reported earlier by Reynolds et al. (45). This is in direct contrast to our studies in the mouse, where the administration of large loads of glutamate leads to a massive hypothalamic lesion (19,45). These data suggest that either the monkey is insensitive to glutamate toxicity or a glutamate metabolite formed by the neonatal mouse, but not the primate, may be the cause of the rodent lesion.

In order to examine primate metabolism of glutamate, three neonatal monkeys were administered a dose of labeled MSG in water (1 g/kg body weight; 10 ucuries ^{14}C) by stomach tube. Sequential plasma samples were obtained and analyzed using the simultaneous amino acid--radioactivity technique of Stegink (49). The administration of 3,4-^{14}C-L-glutamate resulted in significant incorporation of label into glutamate, aspartate, glutamine and ornithine as well as two ninhydrin-negative compounds. The two ninhydrin-negative metabolites of glutamate were isolated and identified using the methods described in our study of glutamate metabolism in the 3-day-old pig (50,52). The compounds were isolated from the plasma by ion-exchange chromatography and high voltage electrophoresis, characterized as single compounds, and identified as glucose and lactate by chemical and enzymatic methods. The data shown in Figure 2 demonstrate the rate of plasma amino acid and metabolite labeling with time. Most of the radioactivity is found

in glutamate and glucose with smaller quantities of label found in aspartate, glutamine, lactate and ornithine.

Little increase in plasma alanine levels was noted either by chemical methods or incorporation of label. These data are in agreement with our observations in the human (51) and the 3-day-old pig (50,52) which suggest that glutamate provides little of the carbon structure of alanine in peripheral plasma after glutamate loading.

The radioactivity in other compounds that are potential metabolites of glutamate was also measured. No detectable radioactivity was found in succinate, pyrrolidone carboxylate, malate, citrate or oxaloacetate following electrophoresis of deproteinized plasma.

Lemkey-Johnston and Reynolds (19) have reported that in addition to the hypothalamus other brain areas such as the tectum, habenular nuclei, subfornical organ, dorsolateral surface of the hypothalamus, denate-hippocampal gyri, cerebral cortex, and in the lower medulla, the nuclei gracilis and cuneatus and area postrema sustain damage following MSG loading. In these structures the lesion is initiated at the surface and radiates inward, suggesting influx of a deleterious agent from the cerebrospinal fluid. We have examined the amino acid composition of the cerebrospinal fluid of one monkey given a 1 g/kg body weight load of MSG (10 μcuries ^{14}C) using the simultaneous amino acid analyzer-radioactivity technique. At the end of 2 hours, samples of the peripheral blood and spinal fluid were

obtained and analyzed. A comparison of the plasma radioactivity-amino acid pattern with that found in the cerebrospinal fluid is found in Table 2. These data demonstrate conclusively that, despite elevated levels of glutamate and aspartate in plasma, neither glutamate nor aspartate entered spinal fluid. Only labeled glutamine appeared at approximately the same level in both plasma and spinal fluid. In direct contrast to the amino acids, glucose and lactate levels were similar in plasma and cerebrospinal fluid reflecting their rapid equilibrium between compartments. These data support the hypothesis of Lemkey-Johnston and Reynolds (19) that the primate responds differently than the rodent to MSG loads.

Neonatal monkeys had substantially higher fasting glutamate levels than did adult animals (Table 3). Our values on the adult monkey range from 5 to 10 μmoles/dl, in agreement with the data of Peters et al. (40). Unusually high plasma glutamate levels were observed in the three youngest animals studied (Table 3). Plasma glutamine levels were normal in these animals, indicating that the elevated glutamate was not due to glutamine degradation. Kerr (23) has reported serum free amino acids values during the development of M. mulatta. His method of assay, which does not quantitate glutamine and glutamate separately, suggest no difference between total plasma glutamine plus glutamate levels in cord blood from those noted in older animals. While it is possible that plasma glutamate levels are elevated at birth and return to normal within a few days, two of the youngest animals

appeared to have decreased ability to metabolize glutamate. Both of these monkeys (MH and MG) had markedly elevated initial glutamate levels and demonstrated abnormal glutamate tolerance curves following a glutamate load. The administration of an oral 1 g /kg body weight MSG load to monkey MH resulted in plasma glutamate levels which rose higher and returned to normal more slowly than those noted in other animals administered this dose (Figure 3). Although MH had not been given radioactively labeled glutamate, it was possible to demonstrate a marked difference in glutamate metabolism. As shown in Figure 4, the conversion of glutamate into aspartate uses much of the same pathway required for the metabolism of glutamate to CO_2, water and energy (tricarboxylate acid cycle) and for its conversion to lactate and glucose (gluconeogenic pathway). The data in Figure 3 clearly demonstrate a marked lag in the conversion of glutamate into aspartate for monkey MH. This is particularly striking when plasma levels at one hour are examined. Plasma glutamate levels are approximately two times those in other animals, while plasma aspartate levels are only one-quarter those found in the other animals. A similar response was noted in monkey MG given a 4 g/kg body weight load of glutamate (Figure 5). Monkey MG showed an enormous increase in blood glutamate when compared to other animals given a similar dose and showed a substantial lag in the conversion of glutamate into aspartate. These data strongly suggest decreased ability to metabolize glutamate in these animals.

In addition to glutamate removed by metabolic processes (Figure 4), a substantial quantity of glutamate is removed from circulation by urinary excretion. Plasma glutamate and aspartate levels are normally maintained at relatively low levels. When substantial elevations in blood glutamate and aspartate occur, renal threshold is exceeded, and these amino acids are not reabsorbed from glomerular filtrate. This is readily demonstrated in Table 4 which shows the radioactivity pattern found in urine samples obtained three hours after glutamate loading (1 g/kg body weight; 10 μcuries ^{14}C). Nearly all of the label is found as glutamate and aspartate with only trace quantities of label present in other metabolites.

While administration of large doses of glutamate resulted in a marked elevation of plasma glutamate and aspartate levels, none of the animals studied, including those with abnormal glutamate tolerance curves, showed evidence of a massive neuronal lesion. It is not known whether these animals exhibited the "micro-lesion" recently reported by Olney et al. (36) since the hypothalamus was not serially thin-sectioned in the manner required to confirm or negate the presence of the newly described lesion.

Although we have measured the response of all plasma free amino acid levels to glutamate load, only plasma glutamate and aspartate data are reported. Small, nonsignificant rises were noted in plasma glutamine, ornithine and alanine levels following glutamate load. As is obvious from the radioactivity data (Figure 2) little glutamate is converted into other amino acids.

Discussion

Studies of amino acid metabolism in man and experimental animals are hampered by the high degree of normal biological variation in physiological fluid levels and by the complex homeostatic mechanisms which control these levels. The use of a radioactively labeled amino acid allows measurement of the net flux of a specific labeled carbon atom but does not differentiate between the specific compounds involved in its metabolism. A number of these problems may be minimized through the use of the simultaneous measurement of radioactivity and amino acid composition using the method of Stegink (49) which distinguishes between incorporation of label from an ingested labeled glutamate into other amino acids or into ninhydrin-negative metabolites.

This method is of greatest value in examining the movement of glutamate and its metabolites into the spinal fluid. The data obtained in the primate indicate that neither labeled glutamate nor aspartate appear in spinal fluid despite elevated blood levels of these amino acids. These data may be of particular importance if the hypothesis of Lemkey-Johnston and Reynolds (19) is correct. Their observations in the mouse suggest the influx of a neurotoxic agent from cerebrospinal fluid. If this hypothesis is correct, the hypothalamus of the primate may be spared because of failure of glutamate or aspartate to enter spinal fluid. Most investigators have found there is little if any net transfer of glutamate across the blood-brain barrier in a

variety of species including the mouse (47), rat (27,47), and dog (17). It is conceivable that glutamate may be able to enter the spinal fluid of the neonatal mouse. It is also possible that a glutamate metabolite is the neurotoxic agent. Our data indicate ready transfer of glucose and/or lactate to the spinal fluid. Thus, if extreme elevations of glucose and lactate occurred in the mouse, deleterious effects could result. In addition, preliminary studies in the mouse indicate a totally different ninhydrin-negative metabolite pattern in the mouse resulting in the production of at least two new major metabolites, not seen in the primate (46).

Conversion of ingested glutamate into lactate and glucose by the liver is reasonable. Portal blood normally contains high levels of glutamate when compared to peripheral blood (52) even in the absence of glutamate loading. This observation indicates that the liver normally converts glutamate into other metabolites. Indeed the labeling of metabolites is logical when one examines the available metabolic pathways (Figure 4). Ingested glutamate is rapidly removed from the portal blood by the liver (50,52). Once inside the liver cell, glutamate enters the mitochondria where it is rapidly converted into α-ketoglutarate and other tricarboxylic acic cycle components, principally malate and oxaloacetate. Oxaloacetate remains within the mitochondria, while malate is able to diffuse out. Mitochondrial oxaloacetate may be transaminated to aspartate which can be transferred to peripheral blood resulting in labeled aspartate. Cytoplasmic malate is converted into phosphoenolpyruvate. Phosphoenolpyruvate may be metabolized in a variety

of ways depending upon the energy or oxidation-reduction status of the liver cell and its precise hormonal balance. In these studies, most of the phosphoenolpyruvate is converted into glucose, while smaller quantities pass through pyruvate into lactate. Since mitochondria have sufficient quantities of α-ketoglutarate available for ATP synthesis, it is reasonable that the conversion of pyruvate into acetyl-CoA for oxidation in the mitochondria is decreased, with the majority of phosphoenolpyruvate being converted into glucose, along with smaller quantities converted into lactate. The incorporation of glutamate into ornithine, citrulline and arginine occurs to some degree indicating minimal conversion of glutamate into glutamate semialdehyde and subsequently to ornithine.

Conversion of glutamate into alanine does not appear to occur at a rapid rate. In view of the utilization of phosphoenolpyruvate for glucose synthesis, there is little indication that alanine formation would be favored by the liver. Our data indicate the presence of only trace quantities of α-ketoglutarate and pyruvate in the plasma after the 1 g/kg load. No detectable radioactivity was noted in plasma citrate, oxaloacetate, succinate, fumarate or malate by either high voltage or simultaneous amino acid analyzer techniques.

Previous experiments from other laboratories indicate that ingested glutamate appears in the portal blood as alanine (26,29,30,41). Our data obtained from lactating women (51), the 3-day-old pig (50,52), and those reported for the neonatal monkey in this paper do not directly support this concept.

Little, if any, ingested glutamate appears in the peripheral plasma as alanine, as evidenced by both chemical and radioactive data. It is possible that a portion of the administered glutamate is converted into alanine during passage through the intestinal mucosa prior to entering portal circulation. This alanine could be the source of the labeled glucose in peripheral plasma, since alanine is a favorite gluconeogenic substrate of the liver (24).

We were surprised to note the unusual glutamate tolerance curve observed in the two neonatal monkeys having markedly elevated initial glutamate levels. All of the available maturational studies in the primate (23), the human infant (10,16,21), including the low-birth-weight infant (11,20), fail to report an increase in plasma glutamate levels during the neonatal period. Thus, we are unable to determine if these two animals represent a normal maturational phenomena or whether they represent an unusual maturational lag in an enzyme system for glutamate metabolism which happened fortuitously two times in the course of this study. Certainly the phenomenon of a maturational lag in enzyme systems metabolizing amino acids is well known for the human infant, particularly those of low-birth-weight. Specific examples of delayed maturation induction include: the delay in enzyme systems catabolizing tyrosine, resulting in the transient tyrosinemia of the premature (5,11); the lag in the development of a part of the phenylalanine hydroxylase system resulting in transient hyperphenylalanemia (4,53); and the lag in the cystathionase synthetase (54) which makes cysteine an essential

amino acid for the human fetus and premature infant (48).

The most pressing problem facing investigators is the need to resolve the conflicting reports of glutamate toxicity in the primate and contrast these results to those obtained in the mouse. Little is known about the levels to which plasma glutamate must rise to cause either type of lesion. Because of this, it is difficult to directly compare toxicity during oral administration to that when subcutaneous or intraperitoneal injection is used. With oral administration, glutamate enters the gut and is carried by portal circulation directly to the liver. Portal glutamate levels are normally high compared to peripheral levels (52) and the liver converts a sizeable quantity of glutamate into glucose and lactate for release to circulation. During subcutaneous injection of glutamate, glutamate enters the circulation without first passing through either the gut or the liver, obviously resulting in markedly increased blood glutamate levels . It would be expected that an injected dose of glutamate would lead to higher blood glutamate levels than would an oral dose.

Few reports have appeared detailing plasma glutamate levels during toxicity studies of glutamate. Olney et al. (36) reported data from five monkeys administered glutamate. Unfortunately the paper chromatographic method used for analysis was not appropriate since glutamine present in the plasma and red cell is converted into glutamate and pyrrolidone carboxylate resulting in artificially elevated glutamate levels. Plasma glutamine levels are approximately five to ten times greater than plasma

glutamate levels, and the conversion of glutamine into glutamate makes the method of little use for quantitating glutamate levels. In addition, the red cell contains four times as much glutamate as plasma along with a large quantity of glutamine. Thus values for total blood glutamate are difficult to interpret. However, even considering these analytical shortcomings, it is obvious that criticism of the data of Reynolds et al. (45) is not valid. Olney et al. (36) suggested that the emetic properties of glutamate caused vomiting in the animals studied by Reynolds et al. (45) with resultant loss of glutamate load. As shown in Table 3, only five of the animals vomited minimal quantities of fluid. One of these animals required redosing because of vomiting early in the experimental period. It is of interest that Olney and colleagues have not published observations of vomiting in their monkeys. The maximum blood levels reported by Olney et al (36) were approximately 680 umoles/dl for whole blood in an animal given MSG subcutaneously, with maximum levels in any animal during oral administration reaching 204 umoles/dl. Considering that the initial total blood glutamate levels reported by Olney et al. (36) ranged from 20 to 136 umoles/dl of whole blood, largely due to inaccuracies in the analytical method, the maximum levels reached during oral administration were markedly below those obtained in our studies. This clearly demonstrates that the inability of Reynolds et al (45) to duplicate the massive lesion reported by Olney et al. (36) is not due to an inadequate level of absorbed glutamate.

The data reported demonstrate the expected correlation between the oral

dose of MSG administered and plasma glutamate levels. The demonstration of elevated plasma glutamate levels in these animals makes the difference between the original report of a primate lesion by Olney and Sharpe (35) and that of Reynolds et al. (45) more striking. This is especially true when it is considered that no monkey, including animals MH and MG who had a marked decrease in their ability to metabolize glutamate, developed the massive hypothalamic lesion reported by Olney and Sharpe (35). Olney et al. (36) note that a critical level of 20 mg% (136 umoles/dl) blood glutamate produces a lesion in the monkey. In evaluating their data it is worth noting that a load of 1 g/kg body weight produced blood glutamate levels of 20 mg% (136 umoles/l) for one-half hour duration (animal D) and resulted in a lesion. Yet, monkey I had baseline glutamate blood levels of 20 mg% prior to any treatment, while control monkey H, (injected with NaCl, 1.2 g/kg) developed blood glutamate levels of 20 to 25 mg% which remained at this level for over 2 hours, reportedly without effect on the animal. This is hardly consistent with a critical level of 20 mg%. In the mouse Perez and Olney (35) have reported maximum plasma glutamate levels of 4000 umoles/dl following subcutaneous injection of MSG at 2 mg/kg. Since normal plasma glutamate levels in the mouse range from 8 to 12 umoles/dl, this represents an increase of approximately 400 fold over normal. In retrospect, it is not surprising to note substantial degenerative processes. Consider the toxic effect to the animal if plasma glucose, sodium or potassium concentrations were elevated 50 to 400 times above normal.

In this connection Lemkey-Johnston, Butler and Reynolds (To be published) have recently found large cerebral and small hypothalamic lesions in the infant mouse following the oral administration of NaCl and sucrose loads equimolar to 4 mg/g loads of MSG. This new finding strongly implicates hyperosmolarity as one causative factor in the production of central nervous system lesions following glutamate administration.

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TABLE 1

LISTING OF ANIMALS STUDIED

Animal	MSG (g/kg)	Age (days)	Weight (g)	Species
MO	0	9	310	M. irus
MC	1	2	450	M. mulatta
ME	1	17	319	M. irus
MH‡	1	0.2	550	M. speciosa
MJ	1	1	370	M. mulatta
MI	1	8	490	M. mulatta
MF	2	4	430	M. mulatta
MA*‡	2	7	445	M. mulatta
MB*	4	14	480	M. mulatta
MP‡	4	6	300	M. irus
MG‡	4	1	510	M. mulatta

* Commercial source

‡ Vomited small amounts of fluid

TABLE 2

COMPARISION OF THE QUANTITY AND DISTRIBUTION OF LABEL IN PLASMA AND SPINAL FLUID FOLLOWING MONOSODIUM GLUTAMATE LOADING OF NEONATAL MONKEYS

COMPOUND	PLASMA		CEREBROSPINAL FLUID	
	counts per minute per ml			
ASPARTATE	638	(3.3)*	0	--
GLUTAMINE	195	(1.0)	112	(2.0)*
GLUTAMATE	10440	(55.0)	90	(1.5)
ALANINE	100	(0.5)	5	(0.1)
ORNITHINE	19	(0.1)	0	--
ARGININE	0	--	0	--
GLUCOSE	5715	(30.0)	4677	(76.9)
LACTATE	1854	(9.7)	1186	(19.5)
TOTAL COUNTS	18961	100	6080	100

*( ) Percent distribution of counts

TABLE 3

EFFECT OF AGE ON FASTING PLASMA GLUTAMATE LEVELS

Monkey	Age (days)	Fasting Plasma Glutamate Concentration (umoles/dl)
MH	0.2	62
MG	1	77
MJ	1	20
MC	2	9
MF	4	15
MP	6	9
MA	7	9
MI	8	15
MO	9	6
MB	14	11
ME	17	8

Range for normal adult animals 5 to 10 um/dl

TABLE 4

DISTRIBUTION OF RADIOACTIVITY IN URINE OBTAINED 3 HOURS AFTER A MSG LOAD

Compound	Counts per min per ml urine	% of total
α-ketoglutarate	473	0.6
glucose	1676	2.1
lactate	1005	1.3
urea	405	0.5
glutathionine (?)	3900	5.0
aspartate	6400	8.3
glutamine	100	0.1
glutamate	62759	81.8
alanine	not detected	
ornithine	not detected	
citrulline	not detected	
arginine	not detected	
Total	76721	100

Figure Legends:

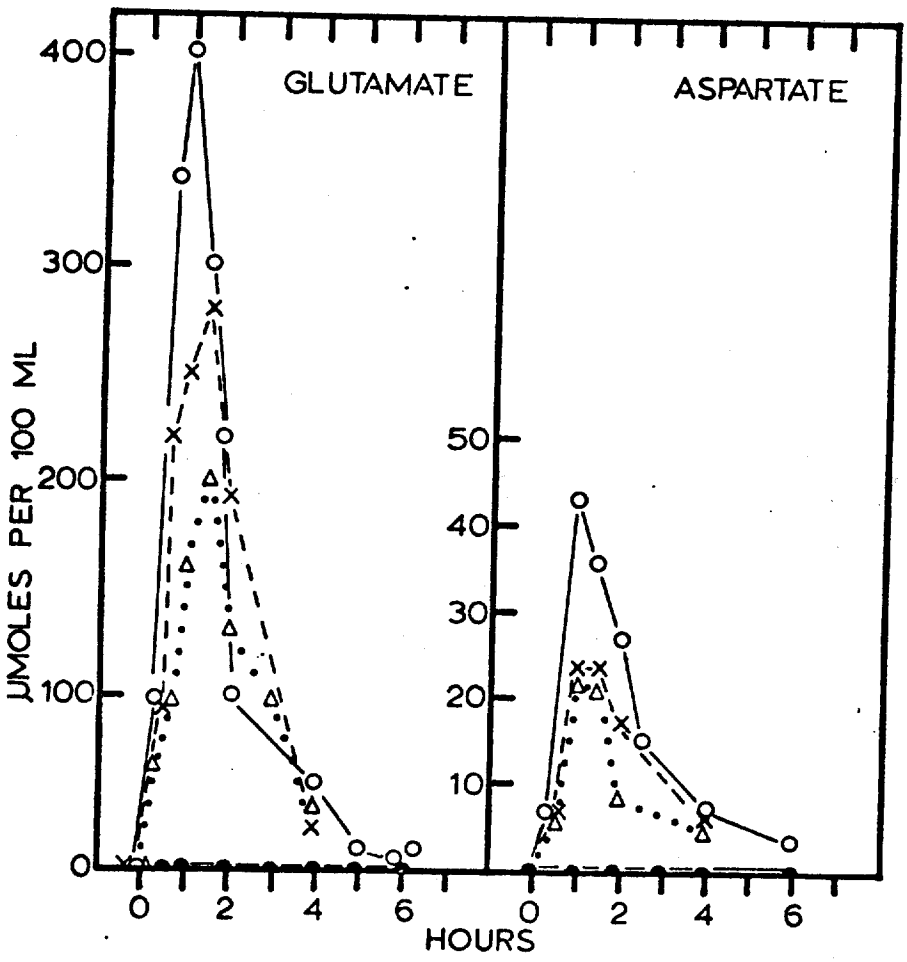
Figure 1. Plasma glutamate and aspartate levels with time following MSG loading: Mean value from 4 animals administered 1 gm/kg body weight (Δ); mean value from 2 animals administered 2 gm/kg body weight (x); mean value from 2 animals administered 4 gm/kg body weight (0); value in control animal administered water (●). Standard deviation not more than 15% of mean.

Figure 2. Plasma distribution of radioactivity in neonatal monkeys (N=3) administered monosodium glutamate in water (1 gm/kg body weight, 10 ucuries 3,4-^{14}C-L-glutamate). Mean values shown, standard deviation did not exceed 15% of the mean.

Figure 3. Plasma glutamate and aspartate levels in neonatal monkeys administered 1 gm MSG per kg body weight: Monkey MH (X); Other neonatal monkeys, mean ± standard deviation (0); control (Δ).

Figure 4. Metabolic pathways available to glutamate.

Figure 5. Plasma glutamate and aspartate in neonatal monkeys administered 4 gm MSG per kg body weight: monkey MG (0); other neonatal monkeys (●).



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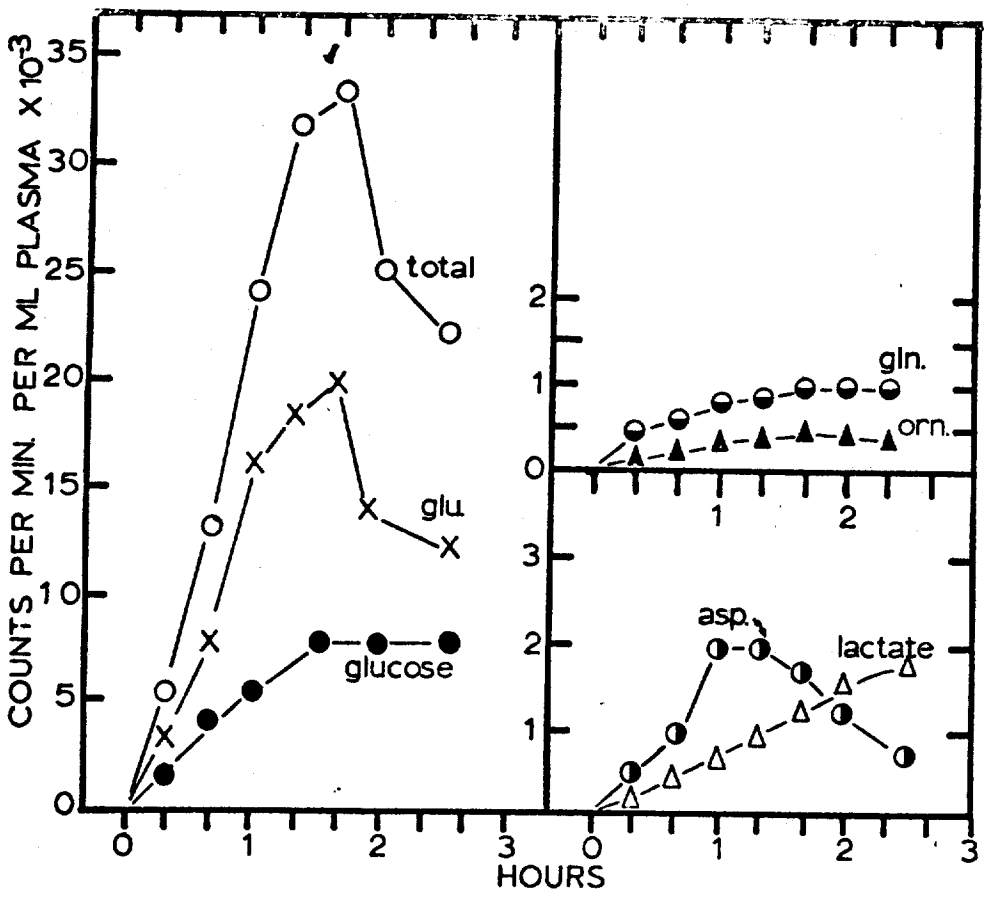

Figure 2. Plasma distribution of radioactivity in neonatal monkeys (N=3) administered monosodium glutamate in water (1 gm/kg body weight, 10 ucuries 3,4-^{14}C-L-glutamate). Mean values shown, standard deviation did not exceed 15% of the mean.

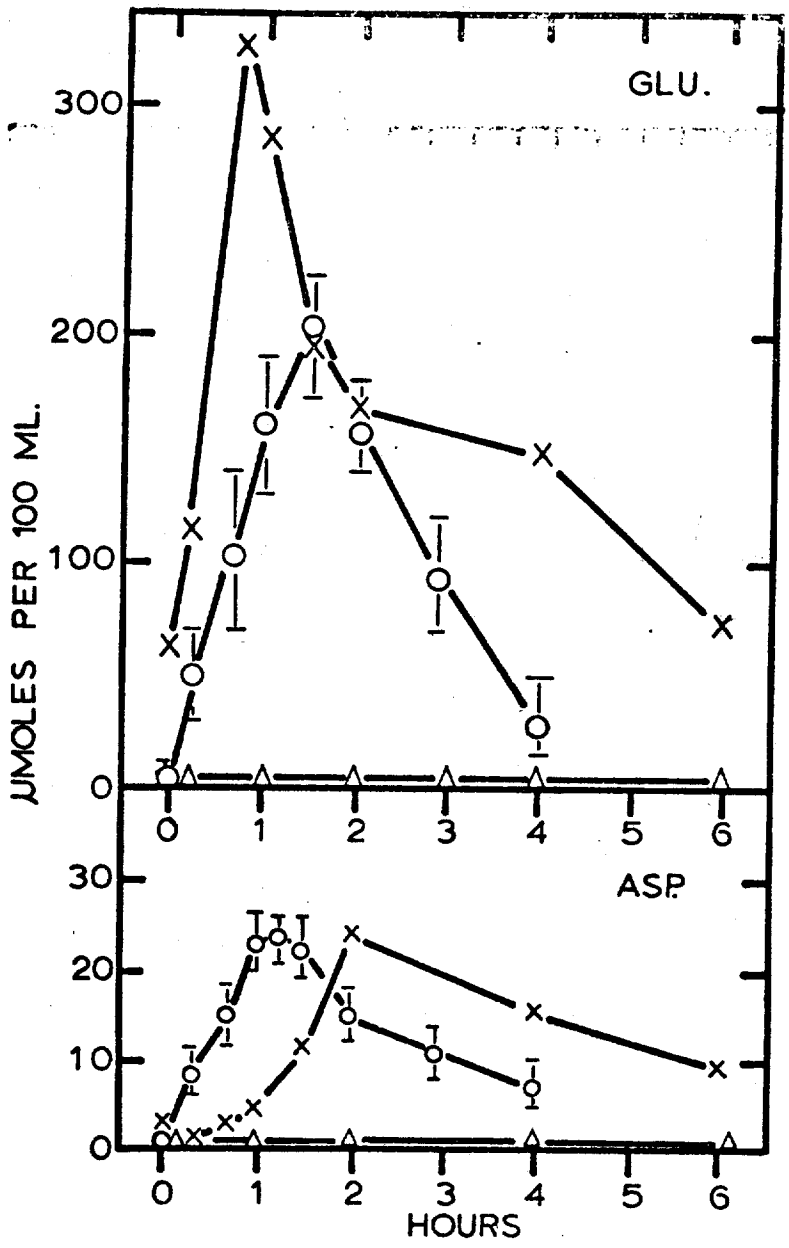

Figure 3. Plasma glutamate and aspartate levels in neonatal monkeys administered 1 gm MSG per kg body weight: Monkey MH (X); Other neonatal monkeys, mean ± standard deviation (0); control (Δ) .

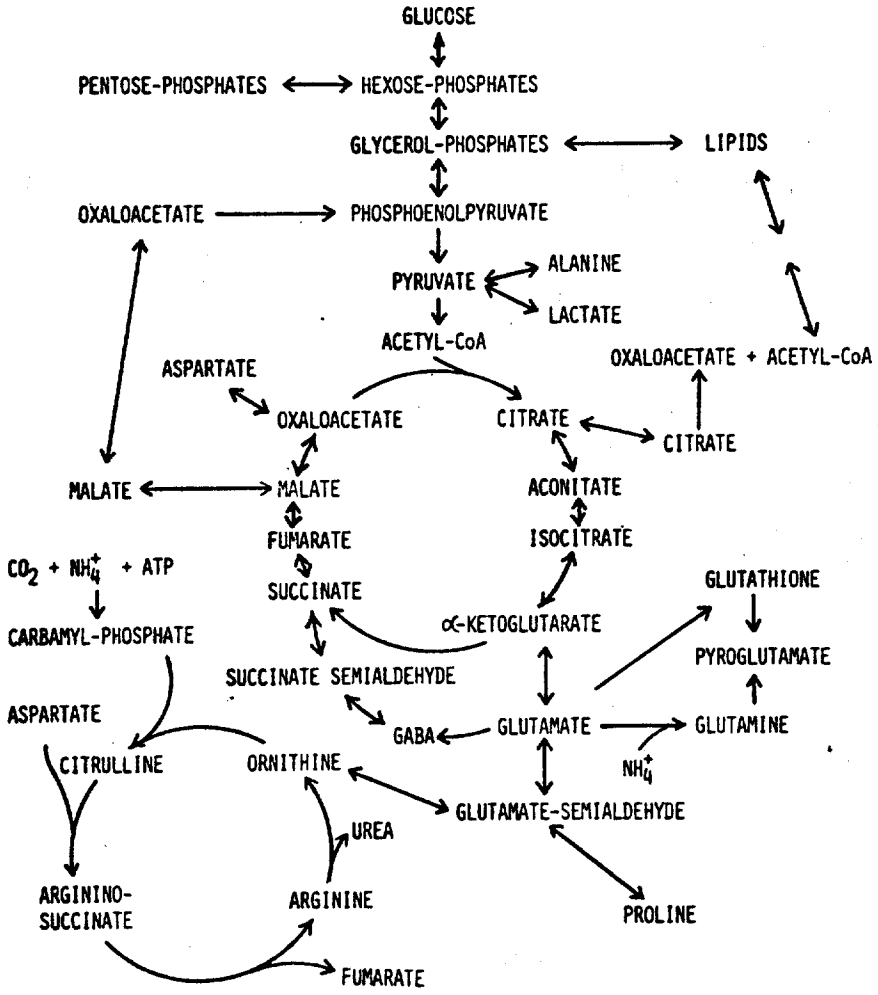

Figure 4. Metabolic pathways available to glutamate.

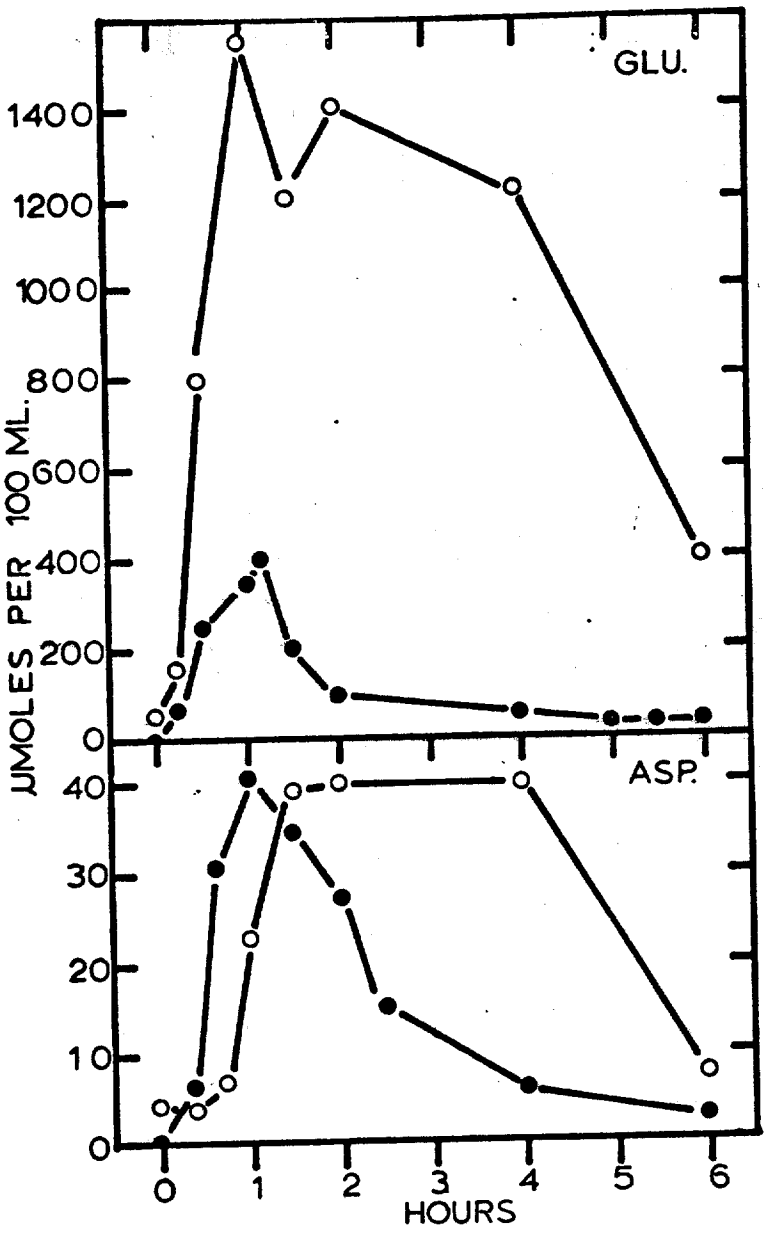

Figure 5. Plasma glutamate and aspartate in neonatal monkeys administered 4 gm MSG per kg body weight: monkey MG (0); other neonatal monkeys (●).

PLACENTAL TRANSFER OF GLUTAMATE AND ITS METABOLITES IN THE PRIMATE

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ABSTRACT:

L-Glutamate-3,4-^{14}C was administered to pregnant rhesus monkeys and serial maternal and fetal plasma samples were analyzed on an amino acid analyzer equipped for simultaneous radioactivity and amino acid analysis. In maternal plasma, 69 to 88% of the readioactivity remained in association with the glutamate, 10 to 22% was converted into glucose, and smaller amounts were converted into lactate, aspartate, glutamine and ornithine during glutamate infusion. In fetal blood however, glucose and lactate accounted for more than 80% of the radioactivity, and less than 2% of the label was found in glutamate in cases where maternal glutamate levels did not exceed 100 μmol/dl. Chemically, when maternal glutamate levels were elevated 10-20 fold (up to 100 μmol/dl) fetal glutamate levels were unchanged. When maternal levels increased to 280 μmol/dl (70 x normal), glutamate did cross the placenta, and fetal glutamate levels were increased but to a far lesser extent (40 μmol/dl) than maternal plasma.

Labeled glutamate administered to the fetus was not transferred to maternal circulation unless fetal plasma glutamate levels were greater than 1000 μmol/dl (normal 4 μmol/dl).

These results indicate that the hemochorial placenta of the primate is virtually impermeable to glutamate, unless extreme elevations of plasma glutamate levels are induced. On the other hand, glutamate metabolites such as glucose, lactate, alanine, ornithine and acetoacetate, but not aspartate, readily traverse the placenta.

"protecting" the fetus from biochemical insults in utero, Kerr and Waisman (21) have pointed out that in some cases the placenta may conceivably potentiate toxic effects. Their conclusions were predicated upon the effects of feeding diets high in phenylalanine to pregnant monkeys and observing that infants born of these mothers had fetal phenylalanine levels considerable higher than those noted in the maternal circulation. Infants born of such mothers were slow to learn simple tasks and social behavior. Based on the observations of Kerr and Waisman (21), Olney (8,11) has suggested that the primate placenta maintains fetal glutamate concentrations at a level two times that of the maternal circulation and proposed that brain lesions could occur in the developing primate embryo in response to increased maternal glutamate levels.

Because of concern as to the safety of MSG ingestion by the pregnant woman, we have studied the extent of glutamate transfer across the hemochorial placenta of the primate.

Materials and Methods:

Surgical Procedures. A total of 8 rhesus monkeys (Macaca mulatta) estimated by physical examination to be in the last trimester of pregnancy were studied. The duration of gestation, estimated retrospectively from standard tables of fetal weight, length and sex for this species (24,25)

Introduction:

Oral or subcutaneous administration of large quantities of glutamate to neonatal (1-11) or adult (8,12) mice results in a variety of neurotoxic effects which include neuronal necrosis, retinal lesions, obesity and neuro-endocrine disturbances. In species other than the mouse, and in the primate in particular, the existence of neuronal necrosis is a subject of considerable controversy (13-19).

The potential danger to the fetus from the administration of large levels of glutamate to the mother is a matter of considerable importance. Available data dealing with glutamate-induced damage to the fetus in utero are conflicting. Lucas and Newhouse (3) reported absence of retinal lesions and no observable abnormalities in the offspring of pregnant mice repeatedly injected with glutamate, while Murakami and Inouye (20) reported brain lesions in the mouse fetus following maternal administration of glutamate. Newman et al. (16) failed to find evidence of glutamate-induced neuronal damage in the newborn progeny of pregnant rhesus monkeys given 4 g monosodium glutamate per kg body weight in their drinking water during the last one-third of pregnancy.

In the pregnant monkey, amino acid concentrations in fetal plasma exceed that of maternal plasma by factors ranging from 1.2 to 2. (21,22). Similar findings have been noted in man, with fetal to maternal ratios for plasma amino acids being highest in the least mature fetus (23).

Although the placenta is usually endowed with the role of

as well as our own experience with several hundred macaque pregnancies at the Primate Breeding Facility, University of Illinois Medical Center, varied from 140 to 160 days. After an overnight fast, the animals were tranquilized with phencyclidine hydrochloride (Sernylan, Parke-Davis Co., Detroit, Michigan), 1 mg per kg, and atropine, 0.1 mg intramuscularly, and anesthetized with halothane by endotracheal inhalation. Polyethylene catheters were inserted into a maternal antecubital vein and into the inferior vena cava through a saphenous vein. The uterus was exposed through a midline abdominal incision, and the anterior and posterior placentas and interplacental vessels were identified by uterine transillumination (26). An interplacental vessel (either a vein or both an artery and a vein) was isolated and catheterized with a silastic T-tube (Extracorporeal Medical Specialties, New Brunswick, New Jersey), permitting sequential sampling of fetal blood without interruption of circulation. After closure of the myometrial incision, a polyethylene catheter was inserted into the amniotic sac at a different site in the uterus. In one experiment catheters were also inserted into the uterine artery and uterine vein. After closure of the abdomen, inhalation anesthesia was discontinued and the animal was maintained in a quiescent state by periodic injections of phencyclidine hydrochloride, 0.5 mg/kg intramuscularly.

Maternal to fetal transfer was examined in 5 animals in which monosodium-L-glutamate (dissolved in distilled water) was infused by a constant infusion pump (Harvard Scientific Corp., Dover, Mass.) into the maternal antecubital vein at a rate of 0.02 g per min. A total of 1 g of glutamate, containing 50 μCi 3,4-^{14}C-L-glutamate, was infused over a course of 60 minutes intc each of 4 animals. Since maternal weights varied from 4.9 to 6.4 kilograms, the total amount administered ranged from 0.16 to 0.22g/kg of body weight. A fifth animal (weight 5 kg) received a total of 2 g of glutamate (75 μCi of radioactive glutamate infused over 60 minutes for a dose of 0.4 g/kg body weight. At intervals of 15 to 30 minutes during and following the infusion, samples of maternal blood, fetal blood and amniotic fluid were collected from the maternal inferior vena cava, the interplacental vein, and the amniotic sac, respectively. In one experiment both arterial and venous samples were obtained from fetal and uterine circulations.

In order to test fetal to maternal transfer of glutamate, 3 animals were studied. In one animal, 0.8 g glutamate containing 10 μCi 3,4-^{14}C-L-glutamat (2.4 g/kg body weight of the fetus) was injected through the uterus and into the fetal chest wall, and maternal plasma amino acid levels were determined at 30 minute intervals for the next 5 hours. In a second animal, the fetus received 0.4 g glutamate containing 10 μCi of 3,4-^{14}C-L-glutamate (1.5 g/kg body weight) injected into the umbilical vein,

and maternal plasma amino acid levels were determined at 30 minute intervals for the following 6 hours. In both of these cases the only fetal blood sample obtained was from the cord at the time of delivery by hysterotomy 5 or 6 hours after fetal injection. In the final animal, 1.2 g of glutamate containing 60 μCi of 3,4-^{14}C-L-glutamate was infused into a previously-catheterized interplacental artery over the course of 60 minutes (5 g/kg body weight of the fetus). Maternal blood and amniotic fluid samples were obtained during infusion and fetal blood samples, maternal blood samples and amniotic fluid samples were obtained during the 2-hour post infusion period.

Each fetus was alive on delivery by hysterotomy at the conclusion of the experiment. Cerebrospinal fluid samples were obtained from the cisterna magna and prepared by the method of Dickinson and Hamilton (27). Plasma and cerebrospinal fluid samples were deproteinized with sulfosalicylic acid (28) and analyzed immediately or stored at -70° until analysis. These conditions prevent conversion of glutamine to glutamate and pyrrolidone carboxylate and loss of cystine. Simultaneous radioactivity and amino acid analysis was done by the method of Stegink (29), a technique permitting detection of both ninhydrin-negative and ninhydrin-positive metabolites. Amino acid analyses were carried out on Technicon NC-1 amino acid analyzers using the Efron buffer system (28).

In order to identify the ninhydrin-negative metabolites of glutamate noted in the primate, adult female non-pregnant monkeys were given large loads of glutamate (1 g/kg body weight, 50 μCi 3-4-^{14}C-L-glutamate). The ninhydrin-negative compounds were isolated from plasma which had been immediately deproteinized either with sulfosalicylic acid (28) or by means of a Diaflo ultrafiltration cell. Samples of deproteinized plasma were applied to an amino acid analyzer column and the eluate collected in 2 ml fractions. The pattern of radioactivity obtained in this manner was identical to that obtained using the simultaneous radioactivity amino acid analyzer technique. Fractional collections were desalted by high voltage electrophoresis techniques which were also used for identification of acidic metabolites (30).

The monosodium-L-glutamate used (International Minerals and Chemical Corp., Skokie, Illinois) assayed at greater than 99.9% glutamate by amino acid analysis and no amino acids other than glutamate were identified. The L-3,4-^{14}C glutamate had a specific acitvity of 0.2 mCi/mmol.

Results:

Maternal to Fetal Transfer

Figure 1 demonstrates the dose-related response of maternal plasma glutamate levels to the glutamate infusion. Maternal glutamate

levels rose in proportion to the amount given and fell rapidly toward normal upon termination of the infusion. In the 4 animals given loads of 0.16 to 0.22 g per kg of body weight and in which maternal glutamate levels did not exceed 100 μmol/dl (20 x normal), fetal glutamate levels were unaffected. However, in the animal infused with 0.4 g/kg body weight, maternal plasma glutamate levels reached 280 μmol/dl (70 x normal) and quantities of glutamate were transferred to the fetal circulation, increasing fetal levels to 44 μmol/dl at 45 minutes into the infusion. Fetal levels then declined so that at the end of the infusion (60 minutes), the glutamate level was 18 μmol/dl, despite a simultaneous maternal level of 250 μmol/dl.

Figure 2 shows the absolute chemical levels of the various amino acids readily derived from glutamate in the maternal and fetal blood in the animal infused at 0.22 g glutamate/kg body weight. As expected, maternal glutamate levels rose rapidly during the infusion reaching levels approximately 20 times baseline values. Upon termination of the infusion, maternal glutamate levels fell to normal within an hour. A small decrease in maternal glutamine and alanine levels occurred during the early part of the infusion, but levels returned rapidly to normal. As expected, a slight rise in maternal aspartate level also occurred. Maternal levels of arginine, proline, ornithine and other amino acids were not affected. In contrast to

maternal circulation, no detectable increase in the levels of glutamate or aspartate occurred in fetal plasma, and fetal levels of alanine, arginine, proline and ornithine were also not affected. Fetal glutamine levels dropped slightly during maternal infusion of glutamate and showed a slight transient elevation upon termination of maternal infusion. Amniotic fluid free amino acid levels were not significantly altered either during maternal infusion or during the post-infusion period. Urine obtained from the fetus at delivery contained normal amounts of glutamate, indicative of lack of placental transfer.

Although moderate elevation of the maternal glutamate level had no effect on the fetal level, radioactivity was transferred from mother to fetus, as illustrated in Figure 3 where arterial and venous values for uterine and fetal blood are given. There was a delay in the transfer of radioactivity from maternal to fetal plasma so that maximal fetal levels were not reached until the cessation of the infusion, although relatively constant levels were maintained for at least 90 minutes thereafter. Little difference in radioactivity was noted between arterial or venous blood on either the maternal or the fetal side of the placenta.

The use of radioactive-labeled glutamate allows the measurement of the net flux of a specific labeled carbon atom but does not

differentiate between the specific compounds involved in its metabolism. This problem was largely eliminated in this study by employing a technique permitting simultaneous analysis of physiological fluid samples for: (a) amino acid composition, (b) the quantity of label found in each amino acid, and (c) the quantity of label in various ninhydrin-negative metabolites derived from these labeled amino acids (29). A typical maternal plasma profile is shown in Figure 4. Glutamate infusion resulted in significant incorporation of label into plasma glutamate, asparate, glutamine, and urea as well as two ninhydrin-negative compounds indicated in Figure 4 as compounds 1 and 2 later identified as glucose and lactate, respectively.

The radioactivity profile of glutamate-derived compounds found in maternal and fetal plasma and amniotic fluid in the animal receiving 0.22 g/kg is illustrated in Figure 5. During glutamate infusion most of the radioactivity in the maternal plasma was present as glutamate, with smaller quantities found as aspartate, glutamine and alanine. Substantial quantities of two ninhydrin-negative compounds identified as glucose and lactate were also noted. Upon termination of the infusion, plasma glutamate levels declined rapidly, leaving glucose and lactate as the major radioactive

compounds. In fetal plasma no radioactivity was detected in either glutamate or aspartate. Small quantities of label were found in alanine and glutamine, appropriate to the concentration gradient for most amino acids maintained toward the fetal circulation by the placenta,although absolute chemical values of these amino acids were not elevated (Figure 2). Amniotic fluid glutamate and aspartate pools were not labeled although substantial quantities of lactate and glucose accumulated. Fetal urine obtained at delivery showed no radioactivity present as glutamate or aspartate. The major radioactive compounds present in fetal urine were glucose and lactate, along with trace quantities of label in alanine and glutamine.

The non-ninhydrin reacting metabolites were isolated from maternal blood and identified by previously-described techniques (31). These compounds were isolated from amino acid analyzer column eluates, purified by high voltage electrophoresis, characterized as single compounds, and identified as glucose and lactate by chemical and enzymatic methods. No substantial quantities of radioactivity were found in succinate, pyrrolidone carboxylate, malate, citrate or oxaloacetate. When large quantities of plasma were applied to the high voltage electrophoresis system, very small quantites of label were noted in pyruvate and α-ketoglutarate.

These data demonstrate that placental transfer of glutamate was negligible under conditions in which maternal plasma glutamate levels were increased as much as 20 times normal. Above this level there existed a threshold value for maternal glutamate levels, which when exceeded allowed some degree of transfer. However, as illustrated in Figure 6, little effect was noted upon the plasma levels of the major glutamate-derived amino acids even in the presence of the maternal glutamate levels 70 x normal.

A small increase in maternal aspartate levels occurred during the infusion although glutamate conversion to aspartate is considerably less than that occurring when glutamate is administered by the oral route (32-34). Upon cessation of the infusion, aspartate levels returned to normal. Just prior to the termination of the infusion, an increase in the urea cycle amino acids, ornithine and arginine (but not citrulline) was noted. This increase was transitory and levels were normal at the next sampling time. In the fetal circulation a plasma glutamate level of 45 μmol/dl was noted at 45 minutes into the infusion. However, at 60 minutes, although the infusion had just terminated and maternal levels were still extremely high (250 μmol/dl), fetal levels had dropped to 18 μmol/dl. This suggests that the maternal level exceeded 280 μmol/dl earlier in the infusion, and that as the maternal level dropped below this value, placental transfer of glutamate ceased. Fetal levels of other amino acids were essentially unchanged, except for aspartate which was slightly elevated.

Evaluation of the radioactivity profile of glutamate metabolites in the animal infused at 0.4 g/kg body weight, illustrated in Figure 7, confirm the absolute chemical values shown in Figure 6. During the infusion almost all of the radioactivity in the maternal plasma was present as glutamate, with much smaller quantities present as glucose, lactate, aspartate and glutamine. Upon termination of the infusion, radioactivity present as glutamate and aspartate disappeared more rapidly than glucose, lactate and glutamine. In the fetal circulation, the radioacitivity present as glutamate correlated closely with the chemical levels, indicating that the decline in fetal glutamate level during the latter portion of the infusion was real. No differences in glutamate metabolite patterns were noted between animals infused at the lower and higher levels, with glucose, lactate, and glutamine containing virtually all of the radioactivity found in fetal plasma.

Fetal-maternal transfer

Reports (35,36) of infants exhibiting hyperglutamic acidemia led us to raise the question of the extent of the placental transfer of glutamate from fetal to maternal circulation in the event of an enzymatic defect in glutamate metabolism in utero. In our two experiments in which glutamate (with added tracer) was injected into the umbilical

vein or the fetus itself in single doses of 1.5 and 2.4 g/kg fetal weight, the maternal plasma failed to demonstrate an increase in the chemical level of any amino acid. Only radioactive glucose and lactate were noted in the maternal circulation . The glutamate level in cord blood samples obtained 4 to 6 hours after administration was normal. However, amniotic fluid glutamate levels were still elevated (18 μmol/dl) in comparison with pre-injection values (3 to 5 μmol/dl). In addition, fetal urine contained enormous quantities of glutamate, suggesting that fetal plasma glutamate levels had been markedly elevated during some portion of the post-injection period.

Figure 8 contains chemical values in maternal and fetal plasma and amniotic fluid following infusion of glutamate (5 g/kg fetal body weight) with 60 uCi 3,4-^{14}C-glutamate to the intrauterine fetus over 60 minutes. Fetal glutamate levels rose to concentrations 400 times the pre-infusion concentrations and amniotic fluid levels behaved similarly. Fetal aspartate levels were raised to 140 times control values. Despite the extreme elevation of fetal glutamate, relatively little glutamate reached maternal circulation. With cessation of the infusion, fetal plasma glutamate levels rapidly dropped below 1000 μmol/dl, and transfer to the maternal circulation apparently ceased. Despite fetal plasma aspartate levels 140 times normal, no increase in maternal plasma aspartate level was detected. Although small increases in fetal plasma glutamine and

alanine levels occurred, no effect upon maternal levels was noted.

The data in Figure 9 indicate that glutamate metabolism is considerably more complicated with fetal infusion than with maternal. This may be due to the enormous quantities given relative to pool size and/or to the storage and recycling of label from the amniotic fluid. Radioactivity in the fetal circulation was found largely as glutamate with smaller amounts of aspartate, lactate, glucose, glutamine and a compound tentatively identified as acetoacetate. A rapid drop in maternal glutamate radioactivity followed cessation of infusion. Again a lack of aspartate transfer was noted.

Comparison of radioactivity profiles in the physiological fluids of the fetal monkey at the termination of the experiment is shown in Table I. Despite elevated levels in fetal plasma, little radioactivity from either aspartate or glutamate is found in the fetal spinal fluid, although glutamine, glucose, lactate and acetoacetate are found in approximately equal quantities in both fluids. Amniotic fluid levels further demonstrate the plight of the fetus in utero. One major mechanism used by the adult organism to remove glutamate following load is urinary excretion (28,31). Normal plasma glutamate levels range from 3 to 10 μmol/dl, and glutamate has a low renal threshold so that marked increases in plasma levels lead to rapid excretion. However, if the fetus clears large quantities of glutamate by urinary excretion,

it goes into amniotic fluid, only to be swallowed by the fetus. Indeed, it is this recycling of label which may account for the more complicated metabolite pattern noted in fetal plasma.

Absolute chemical values found in physiological fluids at the beginning and termination of the experiment are shown in Table II. In the fetal plasma, the greatest proportionate change was noted in glutamate and aspartate, as would be expected on the basis of the radioactivity data. The observation that chemical levels of alanine doubled in the absence of incorporation of radioactive label (Figure 9) is surprising and probably indicates that the carbon structure of alanine comes from other molecules capable of forming pyruvate, with only the amino group coming from glutamate. In the amniotic fluid, most of the amino acids listed increased with fetal glutamate infusion, with the greatest proportionate increases again found in glutamate, aspartate, and alanine. With respect to spinal fluid levels, since pre- infusion samples were not possible, values noted in 2 adult animals are listed for comparison. The increase in spinal fluid amino acids was smaller than that noted with either plasma or amniotic fluid amino acids.

Discussion:

Morse (37) was probably the first to demonstrate that plasma levels of amino acids in the fetus normally exceed those of the mother. Subsequent studies by a number of investigators have indicated that this relationship is maintained by a transport system which: (a) involves

transfer against a gradient, (b) shows discrimination between the D and L-isomers of the amino acids, (c) does not involve binding proteins in the fetal plasma in quantities sufficient to account for accumulation, (d) is competitively inhibited by similar amino acids common to a specific transport site, and (e) can be saturated by increasing concentrations of amino acids. All these data suggest that an active transport process is involved in placental transfer of amino acids (38,39).

In the pregnant monkey, Kerr and Waisman (21) showed that amino acid concentrations in fetal serum exceed those of maternal serum by a factor approximating 1.5 and noted that with the exception of taurine, lysine, alanine and 3-methylhistidine, these disproportionate ratios were achieved on the basis of a decrease in maternal plasma concentrations during pregnancy. In both monkey (21,22) and human (23) the fetal-maternal ratio for amino acids decreases throughout pregnancy. Kerr and colleagues (21,40) pointed out that the transplacental gradient of certain amino acids is maintained even in the face of elevated maternal levels. Thus, increased maternal phenylalanine levels in the primate resulting from ingestion of a high phenylalanine diet produced correspondingly increased fetal levels. Infant primates born of such mothers were slow to learn simple tasks and social behavior, suggesting damaging effects of elevated levels <u>in utero</u> corresponding to the well-documented deleterious effects on human infants born to phenylketonuric mothers (41).

These studies have led to the suggestion that increased maternal levels of any amino acid could present a hazard to the fetus, assuming that each amino acid would be concentrated towards fetal circulation. However, recent data indicate that not all amino acids are concentrated towards the fetal circulation. Gaull and co-workers (42,43) have studied placental transfer of specific amino acids in pregnant women and primates. Maternal infusion of methionine, leucine or ornithine led to the accumulation of these amino acids in fetal plasma. However, infusion of solutions of cystine or cysteine did not result in an increase in their concentration in fetal plasma. From primate studies with ^{35}S-labeled cystine and cysteine, it was concluded that the low fetal plasma concentration for cystine and cysteine was not due to the rapid uptake and/or metabolism of these amino acids by fetal organs or placenta, but rather a transport mechanism based on simple diffusion (43).

Olney (8,11) has suggested that a hazard could exist for the fetus in utero in the event of marked maternal ingestion of glutamate because of unfavorable fetal-maternal glutamate ratios. Various investigators have reported cord-maternal plasma glutamate ratios ranging from 1.58 to 1.8 in the human (21,44,45). In the monkey, Kerr and Waisman (21) reported ratios of approximately 2 for combined glutamine-glutamate levels, since their analyzer did not separate these amino acids. In the present study the fetal-maternal ratios of glutamate ranged from

1 to 1.2, considerably lower than those reported by other investigators. Fetal-maternal glutamine ratios were noted to be 1.5 to 2. Since plasma glutamine levels are usually 10 to 15 times those of glutamate, it is obvious that the combined glutamine-glutamate values of Kerr and Waisman (21) would approximate those of glutamine alone. Thus their data are in agreement with ours. Reliable values for fetal-maternal glutamate ratios are difficult to obtain in the human for several reasons. In view of the demonstration (in the experimental animal) that stress results in glutamate release (46), it is quite likely that labor and delivery may be responsible for some increase in cord glutamate levels. Rooth and Nilsson (47) have suggested that this stress is the cause of a significant arterial-venous difference for glutamate in umbilical cord blood. They correlated this stress with the degree of fetal metabolic acidosis, primarily as a manifestation of delivery and noted it to be more pronounced if labor were complicated. It is possible that stress placed upon our experimental animals could have caused a slight elevation of maternal glutamate levels, with resultant lowering of the fetal-maternal ratio. This appears unlikely, however, since our values for maternal glutamate are no greater than those reported by other investigators.

Our studies indicate that the placenta handles glutamate, and possibly aspartate, differently from most other amino acids. With maternal administration, glutamate appears to cross the placenta only after extreme elevation of maternal plasma levels. Maternal glutamate levels of 100 umol/dl (following glutamate infusion at 0.2 g/kg body weight)failed to produce

any detectable increase in fetal glutamate levels. It seemed likely, however, that there should be a threshold value for maternal glutamate which if exceeded could allow some glutamate transfer to the fetal circulation. To test this point, 0.4 mg/kg body weight glutamate was infused into maternal circulation of one animal. At 45 minutes into the infusion, maternal levels reached 285 μmol/dl and at termination of the infusion, maternal levels were 250 μmol/dl. At these maternal levels glutamate transfer did occur to a limited degree. Interestingly, fetal glutamate levels decreased from 44 μmol/dl (at 45 minutes) to 19 μmol/dl (at 60 minutes of maternal infusion) despite relatively constant maternal levels. This suggests that either the maternal-fetal placental glutamate threshold is somewhat above 250 μmol/dl, and this level was exceeded early in the infusion, or that the infusion of large quantities of "solute" somehow temporarily disrupted the placental membrane allowing some transfer early in the infusion. The results reported here are consistent with the data of Dierks-Ventling et al. (48) in the rat. These investigators injected 1 g/kg body weight of glutamate into the tail vein of the pregnant rat and measured glutamate found in blood of individual pups at various times. Maternal glutamate levels increased from 10 μmol/dl to 350 μmol/dl, but no change was noted in fetal glutamate levels (10 μmol/dl). Unfortunately, these authors did not utilize radioactive glutamate and therefore could not examine labeled compounds derived from glutamate which may have crossed the placenta.

In contrast to the relatively poor placental permeability of glutamate, glutamate-derived metabolites in the maternal circulation appeared to cross the placenta easily. The quantity of radioactivity in glucose was usually slightly higher in the fetal circulation than in the maternal circulation, as was radioactivity in fetal lactate (Figures 5 & 7). The increased levels may reflect concentration of these compounds in the fetal circulation. On the other hand, the more anaerobic conditions of fetal metabolism may cause a greater conversion of labeled glucose to lactate in the fetus.

Conversion of infused glutamate to lactate and glucose is physiologically reasonable. The liver normally converts a large proportion of excess glutamate into other metabolites, and the labeling of metabolites is logical when one examines the available metabolic pathways illustrated in Figure 10. Excess glutamate is rapidly removed from the blood by the liver. Once inside the liver cell, glutamate enters the mitochondria where it is rapidly converted into α-ketoglutarate and other tricarboxylic acid cycle components, principally malate and oxaloacetate. Oxaloacetate remains within the mitochondria, while malate is able to diffuse out. Mitochondrial oxaloacetate may be transaminated to aspartate which can be transferred to peripheral blood resulting in labeled aspartate. Cytoplasmic malate is converted into phosphoenolpyruvate which in turn may be metabolized in a variety of ways depending upon the energy or oxidation-reduction status of the liver cell and its precise hormonal balance. In these studies, most of

the phosphoenolpyruvate was converted into glucose, while smaller quantities passed through pyruvate into lactate. Since mitochondria have sufficient quantities of α-ketoglutarate available for ATP synthesis, it is reasonable to postulate that the conversion of pyruvate into acetyl-CoA for oxidation in the mitochondria was decreased, with the majority of phosphoenolpyruvate being converted into glucose, along with smaller quantities converted into lactate.

Our studies involving fetal administration of glutamate suggest that the threshold for transfer from fetus to mother is even higher than that for mother to fetus. In the two experiments in which the fetus received glutamate at 1.5 and 2.4 g/kg, maternal glutamate levels were unchanged and radioactivity in the maternal circulation was present only as glucose and lactate. Although fetal infusion of glutamate at 5 g/kg did produce chemical and radioactive evidence of some transfer (fetal glutamate level ca. 2000 μmol/dl) transfer apparently ceased during the 3 hours following fetal infusion in which fetal plasma glutamate levels remained in the range of 400 to 1200 μmol/dl. This observation suggests that the threshold for fetal-maternal transfer is in excess of 1000 μmol/dl. In addition to glucose and lactate, an additional metabolite (tentatively identified as acetoacetate) was found in the animal given the largest fetal infusion.

The present study also suggests that aspartate (the other dicarboxylic acid present in plasma) has a similar lack of placental transfer. With

maternal infusion, increases in maternal aspartate caused only a small increase in fetal aspartate levels (Figure 6) and virtually no label detectable in fetal aspartate (Figure 7). Similarly, fetal aspartate levels of 50 to 70 µmol/dl (normal 0.4 to 0.8) in the fetal infusion experiment failed to increase maternal aspartate measured either chemically or radioactively (Figures 8 and 9). Thus, it appears that the so-called "acidic amino acids" such as glutamate and aspartate are generally not transported across the placenta. The sulfonic amino acid, taurine, may also fit this grouping. It has been suggested that the elevated fetal to maternal taurine levels are due primarily to the failure of taurine transport from fetal to maternal circulation after taurine synthesis from cysteine in the fetus (49).

Although Lucas and Newhouse (3) did not observe retinal lesions in the offspring of pregnant mice injected with glutamate, Murakami and Inouye (20) have reported a brain lesion in the mouse fetus following maternal glutamate administration. At the dosage employed in the latter study (5 g/kg body weight), it is entirely possible that some transfer to fetal circulation could occur. Perez and Olney (50) reported blood glutamate levels of 4000 µmol/dl in neonatal mice injected with glutamate at 2 g/kg body weight. Thus, at 5 g/kg maternal dose, the maternal glutamate levels could well exceed the placental threshold to a considerable degree, assuming the mouse placenta has a threshold

similar to the primate. In addition, it is now generally accepted that the neonatal mouse is particularly susceptible to glutamate-induced neuronal damage. For example, Olney et al.have reported some neuronal damage in neonatal mice injected with glutamate-containing solutions (51) in doses we have shown to produce blood glutamate levels of only 40 to 70 μmol/dl (52). However, the neurotoxic effects of glutamate in species other than the mouse are debatable. Although Olney and colleagues have reported damage to the arcuate nucleus of the neonatal primate by high doses of glutamate (18,19), other laboratories have failed to confirm their report of a massive lesion in the arcuate region (13-16). Olney et al. (19) recently redescribed the size and site of the primate lesion, and now report the presence of small 50 to 90 cell lesions in the infant primate administered lower levels of glutamate while reasserting the existence of the more massive lesion originally reported at higher dose levels. They correlate the size of the lesion to the blood glutamate levels reached, stating that a critical blood glutamate level of 136 μmol/dl results in neuronal necrosis (19,54). However, the paper chromatographic method used by Olney et al. (19) to determine glutamate levels was not appropriate, and results in artifactual elevation of blood glutamate levels due to glutamine degradation (53). This makes the data presented by these authors (19) difficult to evaluate. For example, they report a massive neuronal lesion in animals in which blood glutamate levels reached 5 to 6 times baseline levels, while reporting only a small 80 to 90 cell lesion in an

animal in which blood glutamate levels are elevated 10 times baseline levels. Similarly, control animals had blood glutamate levels which exceeded 136 μmol/dl, reportedly without evidence of neuronal necrosis (19). In contrast to their data, Reynolds et al. (13) reported no evidence of massive neuronal necrosis in animals in which we have shown plasma glutamate levels to reach 900 umol/dl (34).

While the issue of glutamate-induced neurotoxicity in the young of species other than the mouse remains controversial, it seems obvious that neuronal damage to the fetus is unlikely in the absence of elevated fetal glutamate levels. In the present investigation, maternal glutamate loads producing plasma concentrations up to 100 μmol/dl did not result in any transfer of glutamate to the fetal circulation. In previous studies of glutamate metabolism we found maximal plasma glutamate levels of less than 30 μmol/dl with oral loads of 0.1 g/kg in adult humans (33), 240 μmol/dl with oral loads of 1 g/kg in infant monkeys (34), and 130 μmol/dl with oral loads of 1 g/kg in neonatal pigs (32). In order to significantly raise the fetal glutamate level, a maternal intake in excess of 1 g/kg would be required, an amount highly unlikely to be ingested as a single dose.

Newman et al. (16) failed to find evidence of glutamate-induced neuronal damage in the newborn progeny of pregnant rhesus monkeys given 4 g monosodium glutamate per kg body weight during the last

one-third of pregnancy. In their studies (16) glutamate was administered in the drinking water, thus the load of 4 g per kg body weight per day was spread over 24 hours. This method of administration would produce blood levels considerably below those expected if the animal was given an acute loading dose of 4 g/kg body weight. In any case, the neonatal primate appears considerably more resistant to glutamate-induced neuronal damage than the neonatal mouse. Reynolds et al. (55) have infused glutamate loads directly into fetal circulation in utero without production of the lesion reported by Olney and Sharpe (18).

The fetus lacks an efficient mechanism for clearance of excess glutamate to maternal circulation. Thus, should a fetal metabolic defect in glutamate metabolism exist in utero, such a fetus might be subject to some hazard from the enormous accumulations of glutamate which would result. This may have occurred in the two children reported to have a defect in glutamate metabolism (35,36).

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TABLE I

Radioactivity Distribution Physiological Fluid Samples
at the Termination of the Fetal to Maternal Transfer Experiment

Counts per min per ml fluid

Compound	Maternal Plasma	Fetal Plasma	Fetal Spinal Fluid	Amniotic Fluid
Glucose	1044	850	650	796
Lactate	509	700	420	898
Acetoacetate		1600	2700	2264
Aspartate	15	4600	162	3990
Glutamine	100	834	810	790
Glutamate	55	62800	486	201064

TABLE II

Composition of Physiological Fluid Samples
at the Beginning and Termination of the Fetal to Maternal Transfer Experiment

umol per 100 ml

Compound	Fetal Plasma		Amniotic Fluid		Spinal Fluid	
	Initial	Final	Initial	Final	Normal Adult N=2	Final
Aspartate	0.4	70.0	0.2	52.8	0.15 - 0.30	2.1
Glutamine	56	67.3	19.0	45.3	40.1 - 51.2	56
Glutamate	3.6	570	2.1	1351	1.15 - 1.85	8.9
Proline	18	25.7	14.5	15	trace	trace
Citrulline	1.6	2.3	0.3	0.2	0.10 - 0.20	0.5
Glycine	49.6	60.4	41	60.1	1.38 - 1.82	3
Alanine	37	73.7	12.3	52.2	2.20 - 4.5	12
Ornithine	5.6	9.6	1.9	4.9	0.17 - 1.19	2.9
Arginine	8.0	12.0	1.1	4.4	1.31 - 1.89	5.1

FIGURE LEGENDS

FIGURE 1. Maternal and fetal plasma glutamate levels during maternal infusion of glutamate at several levels: ▲, 0.15 g/kg body weight (1 animal); x, 0.17-0.19 g/kg body weight (mean of 2 animals); ●, 0.22 g/kg body weight (1 animal); and 0 , 0.40 g/kg body weight (1 animal).

FIGURE 2. Maternal and fetal plasma levels of glutamate derived amino acids during and following maternal infusion of glutamate (0.22 g/kg body weight).

FIGURE 3. Total plasma radioactivity in maternal arterial (o), maternal venous (●), fetal arterial (△) and fetal venous (▲) blood during and following maternal infusion of 3,4-^{14}C-L-glutamate (0.15 g/kg body weight).

FIGURE 4. Typical radioactivity--amino acid analyzer profile of maternal plasma.

FIGURE 5. Radioactivity profile of glutamate metabolites during and following maternal glutamate infusion (0.22 g/kg) in maternal plasma, fetal plasma and amniotic fluid.

FIGURE 6. Maternal and fetal plasma levels of glutamate-derived amino acids during and following maternal glutamate infusion at 0.40 g/kg body weight.

FIGURE 7. Radioactivity profile of glutamate metabolites during and following maternal infusion of 3, 4-^{14}C-L-glutamate (0.40 g/kg body weight).

FIGURE 8. Maternal plasma, fetal plasma and amniotic fluid free amino acid levels following fetal infusion of glutamate (5 g/kg body weight).

FIGURE 9. Radioactivity profile of glutamate metabolites in fetal plasma, maternal plasma and amniotic fluid following fetal infusion of glutamate (5 g/kg body weight, 50 uCi, 3,4-^{14}C-L-glutamate).

FIGURE 10. Pathways available for glutamate metabolism.

FIGURE 1

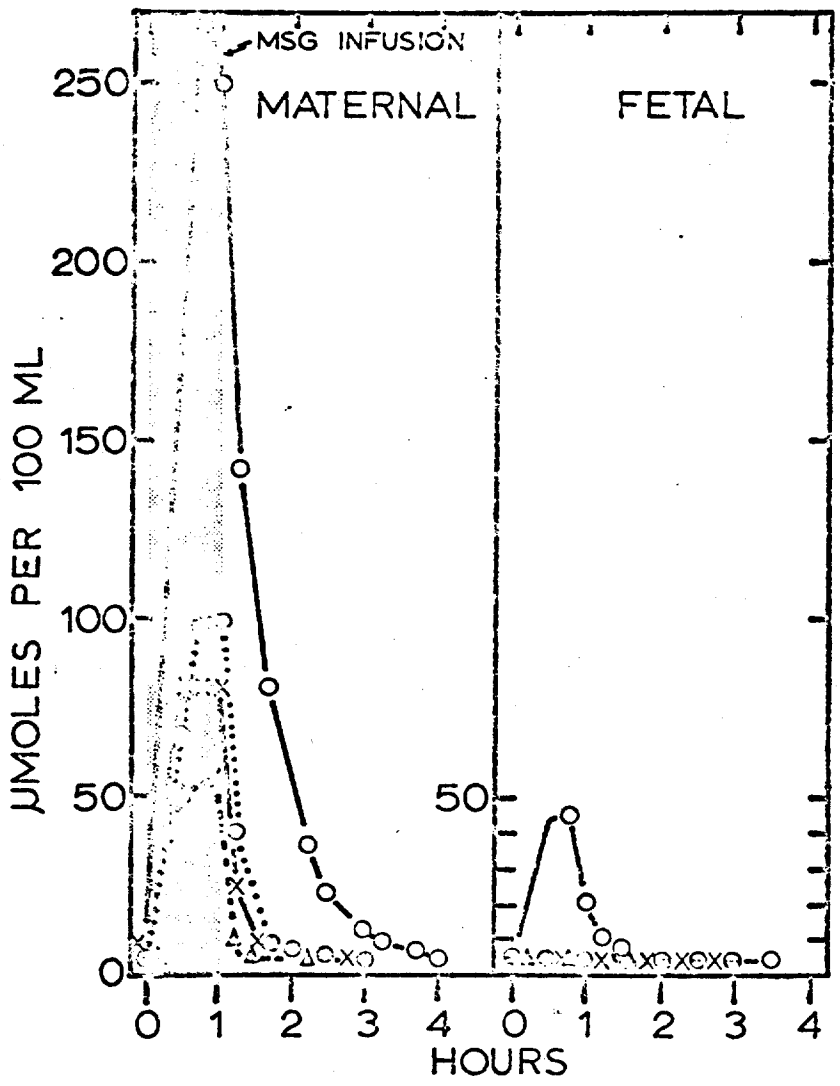

FIGURE 1. Maternal and fetal plasma glutamate levels during maternal infusion of glutamate at several levels: Δ, 0.15 g/kg body weight (1 animal); x, 0.17-0:19 g/kg body weight (mean of 2 animals); 0, 0.22 g/kg body weight (1 animal); and 0 , 0.40 g/kg body weight (1 animal).

FIGURE 2

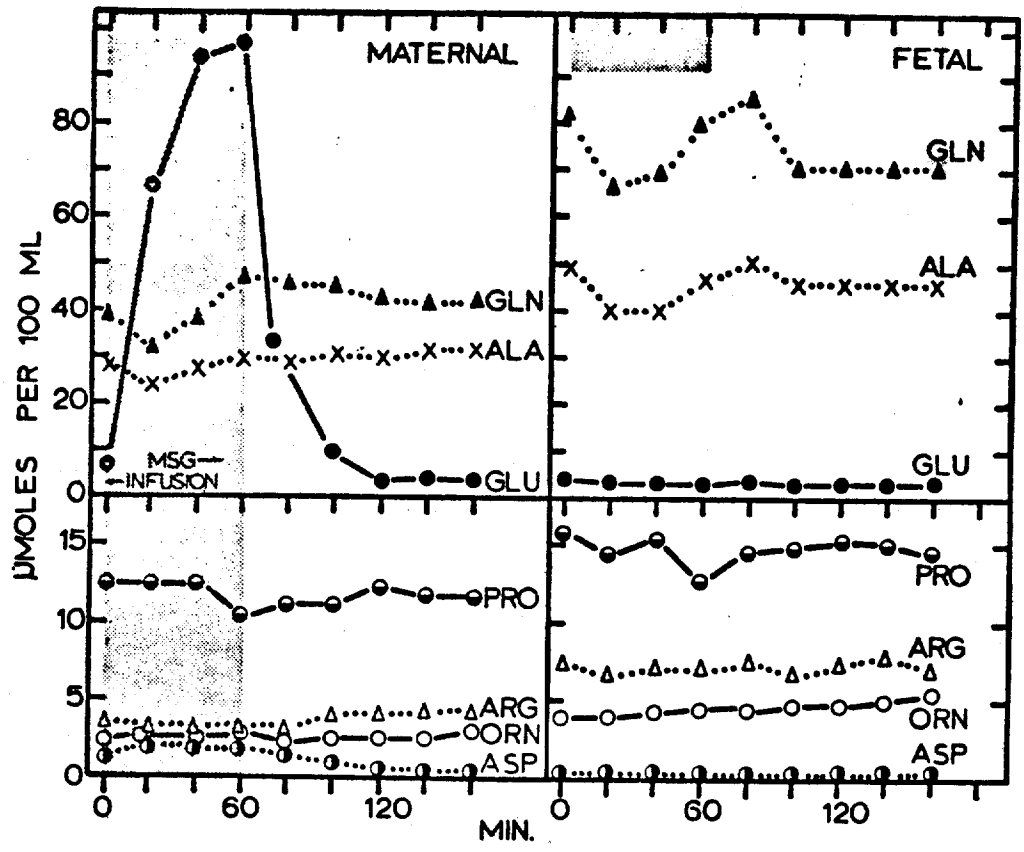

FIGURE 2. Maternal and fetal plasma levels of glutamate derived amino acids during and following maternal infusion of glutamate (0.22 g/kg body weight).

FIGURE 3

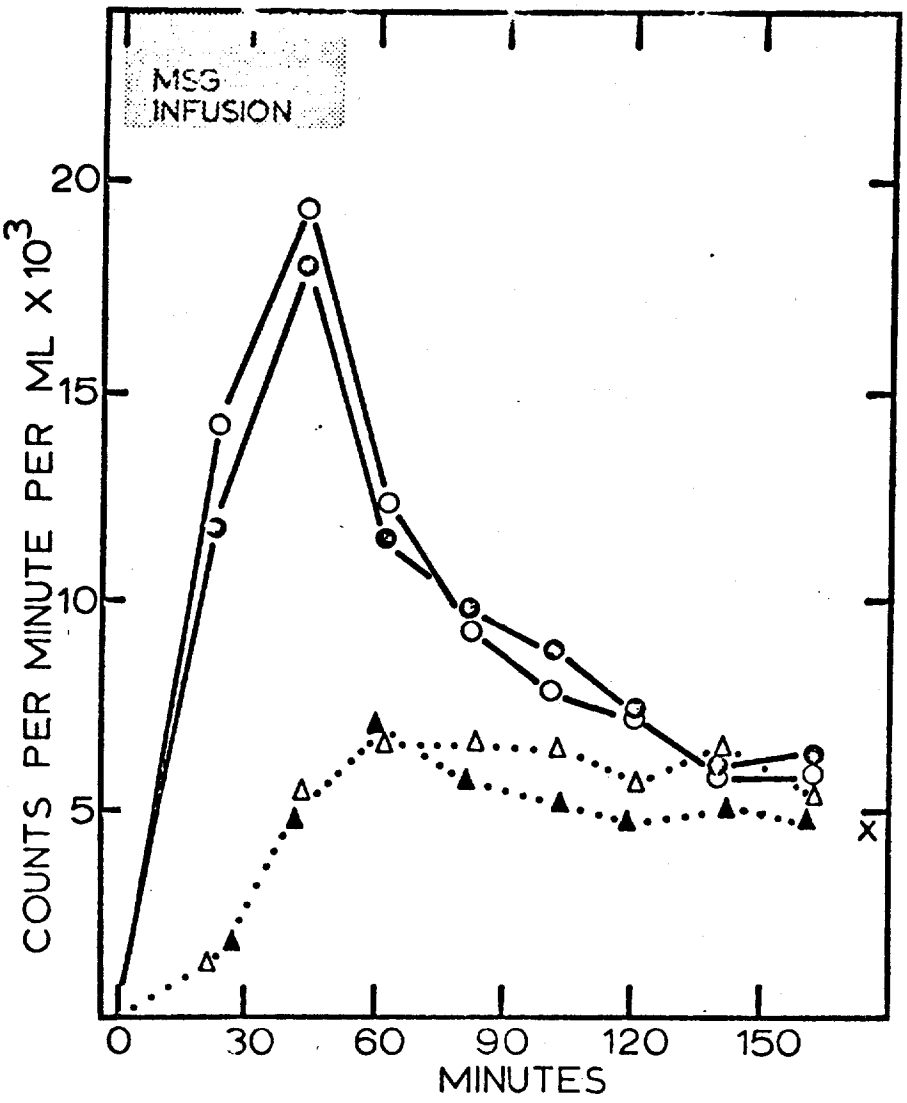

FIGURE 3. Total plasma radioactivity in maternal arterial (o), maternal venous (ɔ), fetal arterial (Δ) and fetal venous (▲) blood during and following maternal infusion of 3,4-^{14}C-L-glutamate (0.15 g/kg body weight).

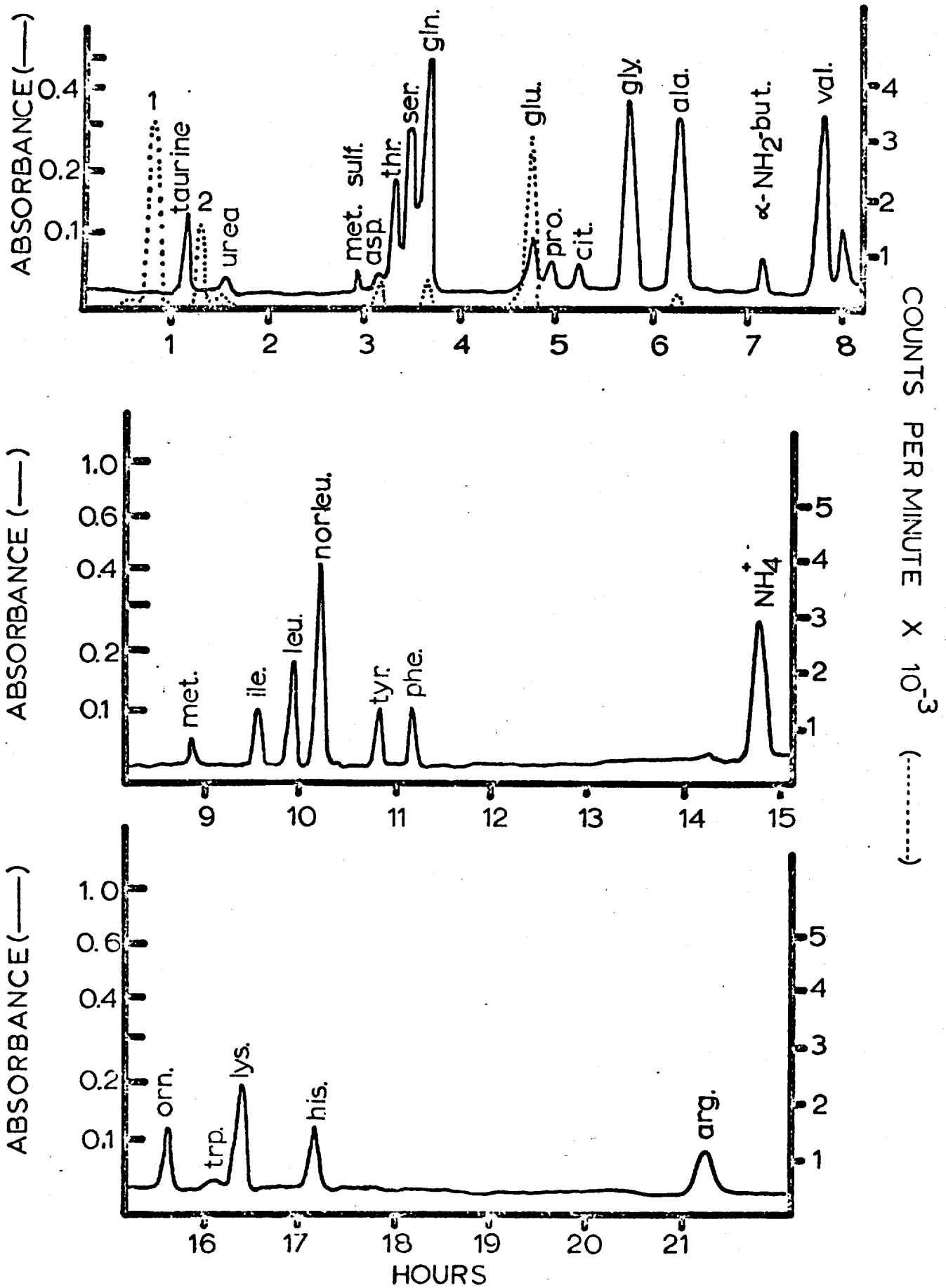

FIGURE 4. Typical radioactivity--amino acid analyzer profile of maternal plasma.

FIGURE 5

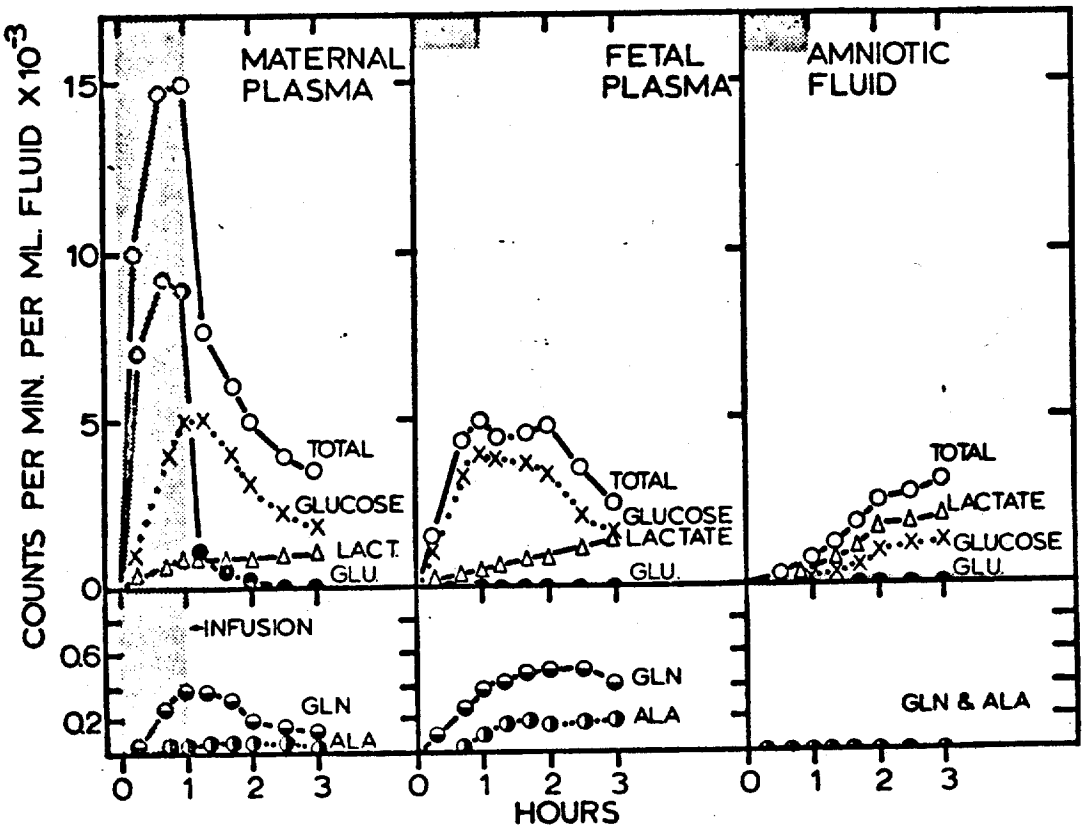

FIGURE 5. Radioactivity profile of glutamate metabolites during and following maternal glutamate infusion (0.22 g/kg) in maternal plasma, fetal plasma and amniotic fluid.

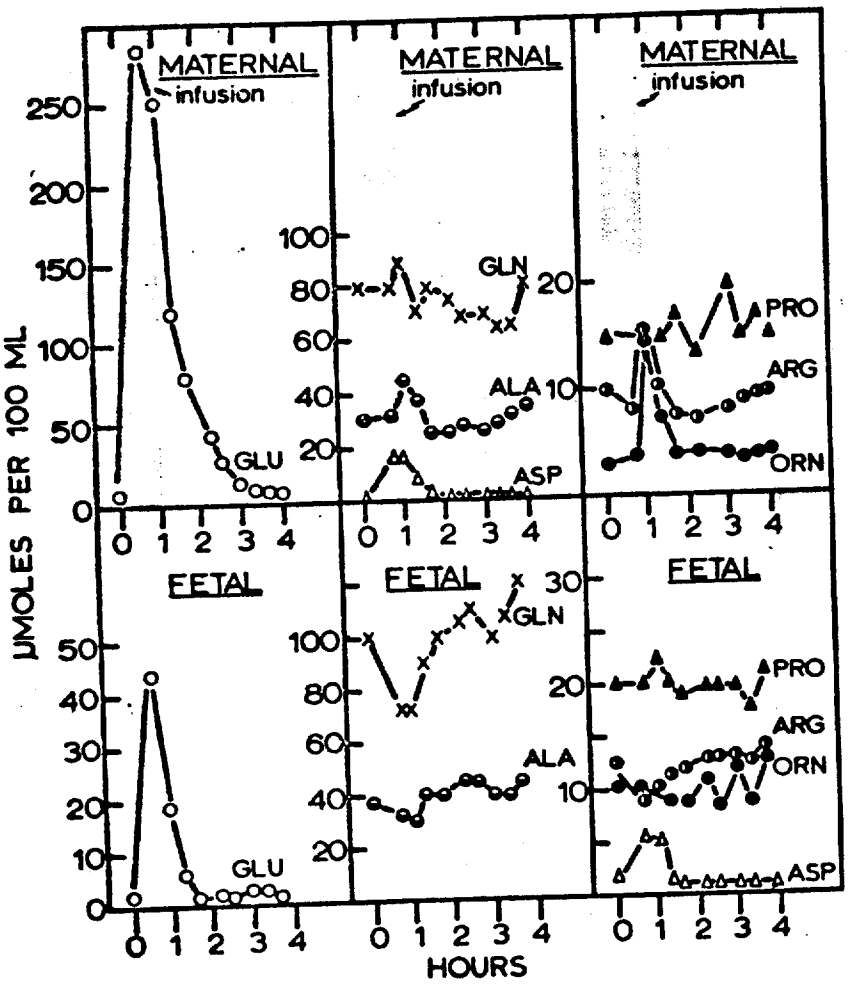

FIGURE 6. Maternal and fetal plasma levels of glutamate-derived amino acids during and following maternal glutamate infusion at 0.40 g/kg body weight.

Figure 7

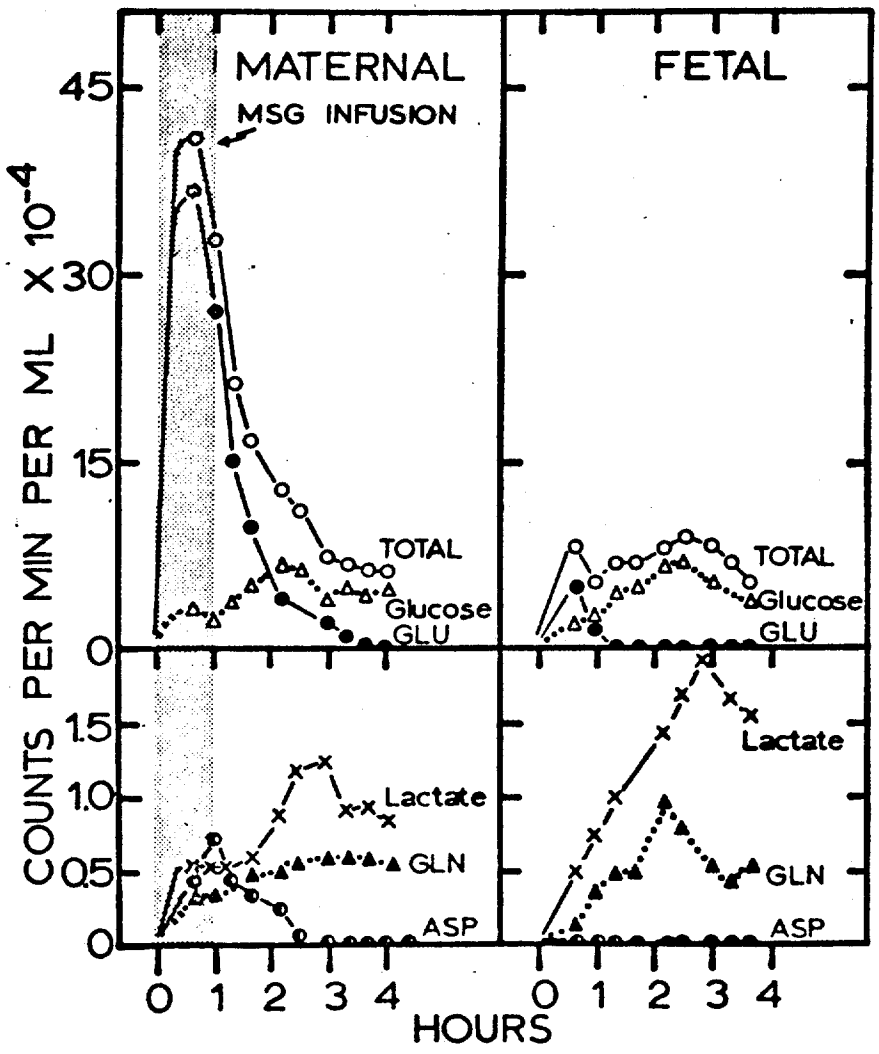

FIGURE 7. Radioactivity profile of glutamate metabolites during and following maternal infusion of 3, 4-^{14}C-L-glutamate (0.40 g/kg body weight).

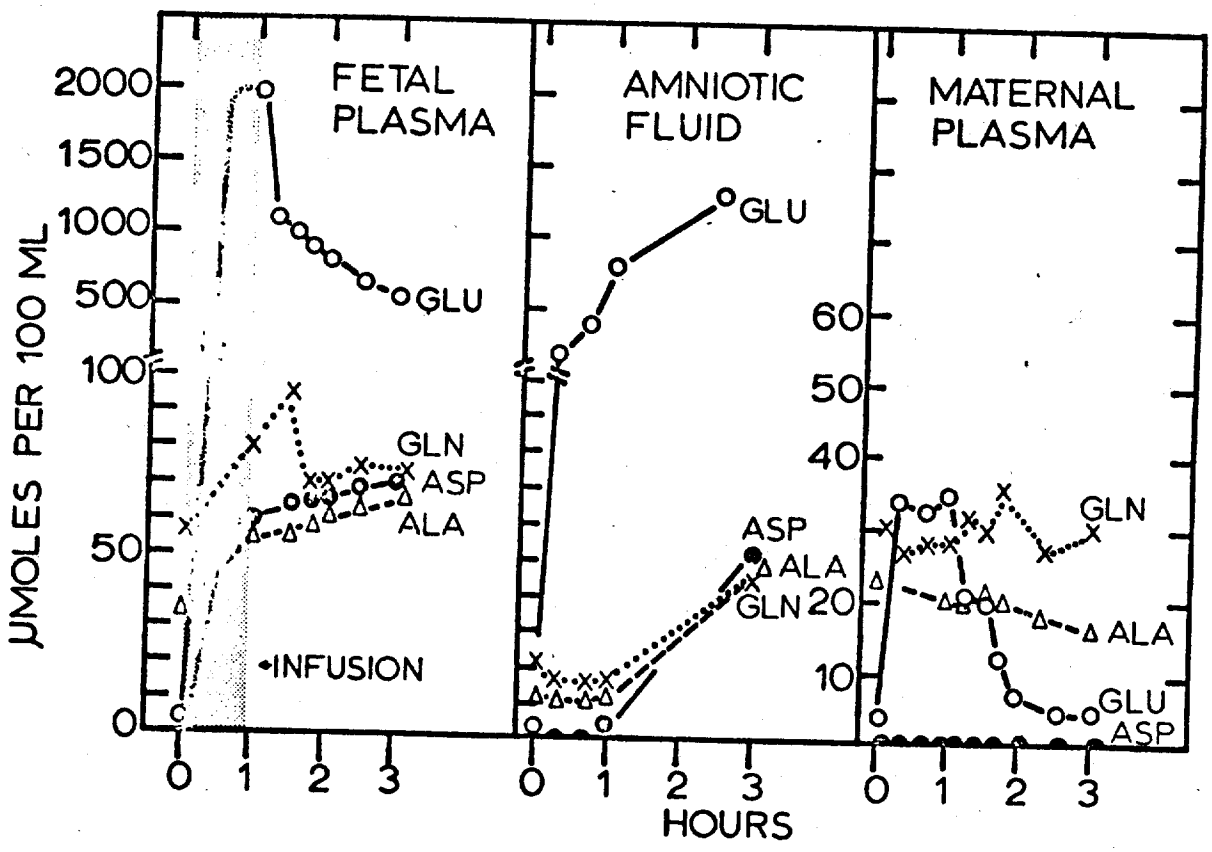

FIGURE 8. Maternal plasma, fetal plasma and amniotic fluid free amino acid levels following fetal infusion of glutamate (5 g/kg body weight).

Figure 9

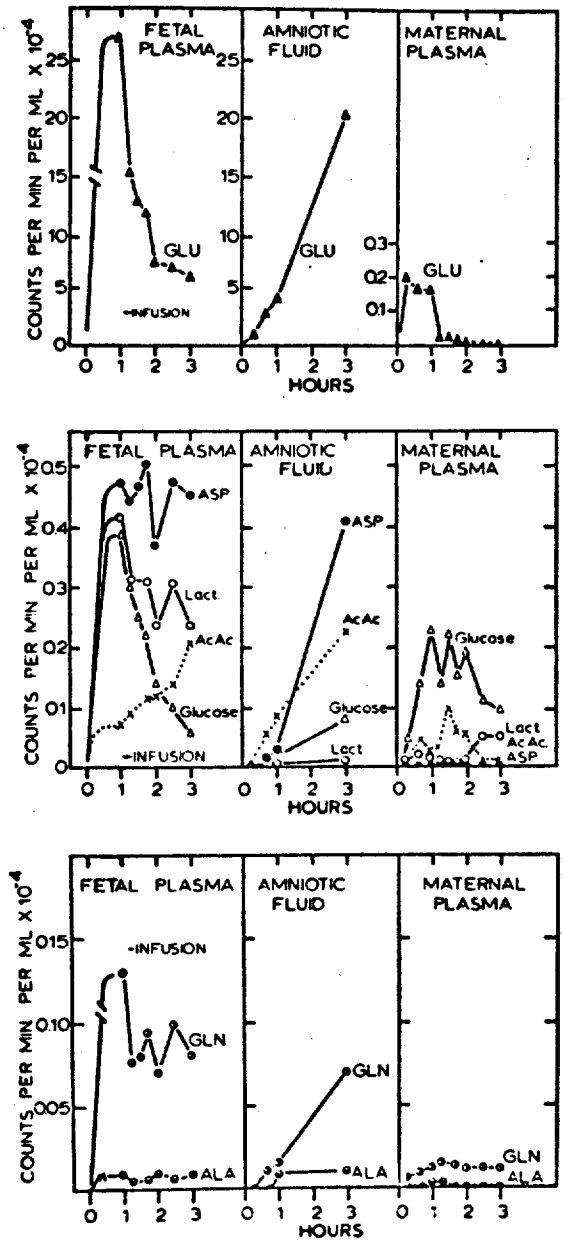

FIGURE 9. Radioactivity profile of glutamate metabolites in fetal plasma, maternal plasma and amniotic fluid following fetal infusion of glutamate (5 g/kg body weight, 50 uCi, 3,4-^{14}C-L-glutamate).

FIGURE 10

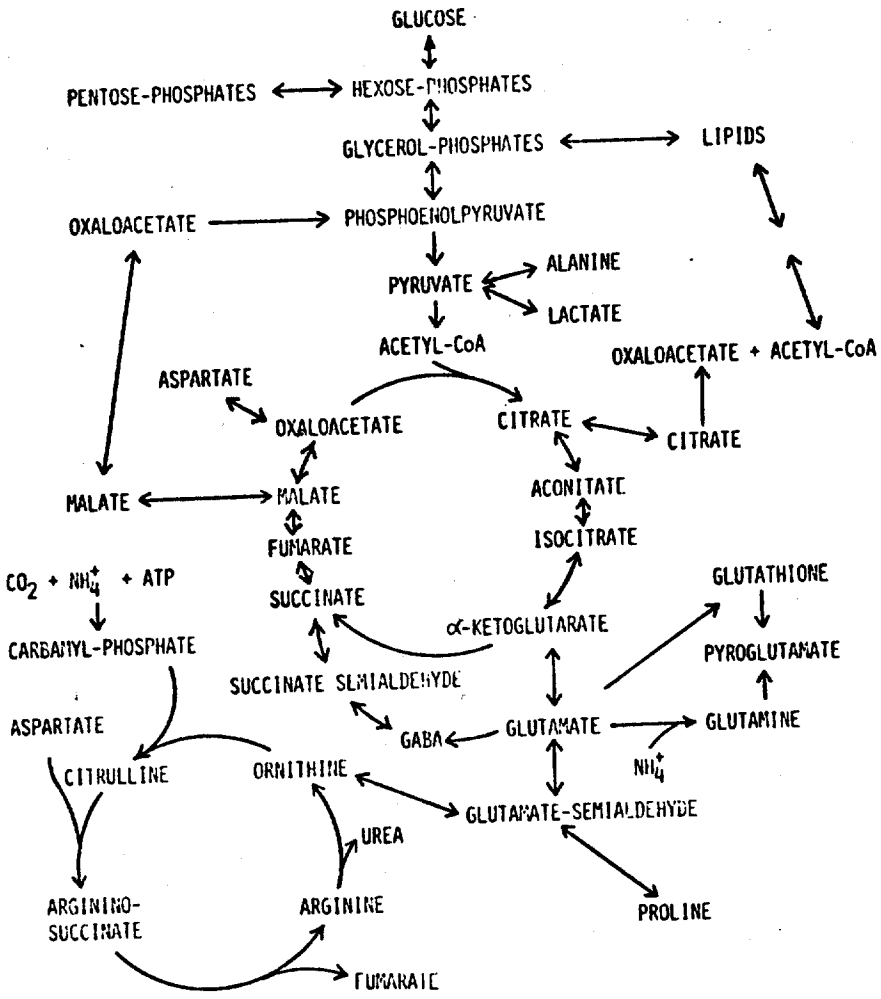

FIGURE 10. Pathways available for glutamate metabolism.